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ADVISORY COMMITTEE ON PESTICIDES

FOOD AND ENVIRONMENT PROTECTION ACT 1985, PART III

Control of Pesticides Regulations 1986

Evaluation of Fully Approved
or Provisionally Approved Products

ZINEB: USE AS A BOOSTER BIOCIDE IN ANTIFOULING PRODUCTS

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Please note that this document reflects the outcome of the 278th Advisory Committee on Pesticides meetings held in September 2000.

The review in 2000 took into account all data that had been made available at the time and that were considered to be of adequate quality.

Data requirements recommended by the ACP and agreed by Ministers as a consequence of the review in 2000 have subsequently been answered by the relevant organisations. These data will be considered further in due course.

Antifouling active substances including Zineb are to be reviewed under the Biocidal Products Directive in the second review group. All relevant data including those generated as a consequence of the 2000 UK review will be taken into account.

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ABBREVIATIONS

ACP	Advisory Committee on Pesticides
AAS	atomic absorption spectroscopy
a.i.	active ingredient
ACDH	alcohol dehydrogenase
ACN	acetoanilide
ADP	adenosine diphosphate
2AF	2-acetylaminofluorine
AFP(s)	antifouling product(s)
AH	aniline hydroxylase
APDM	aminopyrine-N-demethylase
AP	aminopyrine
AR	applied radioactivity
ASA	acetyl salicylic acid
AST	aspartate amino transferase
ALT	alanine amino transferase
BBA	Federal Biological Institute for Agriculture and Forestry (Germany)
bw	body weight
C	carbon
^{14}C	carbon-14, radioactive isotope
^{o}C	degrees Celsius
$CaCl_2$	calcium chloride
$CaCO_3$	calcium carbonate
CAS	Chemical Abstracts Services
CDNB	1-chloro-2,4-dinitrobenzene
CEFAS	Centre for Environment, Fisheries & Aquaculture Science
CEPE	European Confederation of Paint, Printers' Inks and Artists' Colours Manufacturers Association
CHO	Chinese hamster ovary
cm	centimetre
CO_2	carbon dioxide
CoT	committee on toxicity
CS_2	carbon disulfide
Cu_2O	copper I oxide
d	day(s)
2D	two-dimensional
DAB	diaminobenzidine
DEFRA	Department of Environment, Food and Rural Affairs
DIDT	5,6-dihydro-3H-imidazo (2,1-c) 1,2,4-dithiazole-3-thione
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DT_{50}/DT_{90}	time for 50/90 % to degrade
DTLR	Department of Transport, Local Government and the Regions
EA	Environment Agency
EBDC(s)	ethylene bisdithiocarbamate(s)
EBIS	ethylene bisdiisothiocyanato sulfide
EC_{50}	median effective concentration
EDA	ethylenediamine
EDI	ethylene diisothiocyanate

EINECS	European Inventory of Existing Commercial Chemical Substances
ELISA	enzyme linked immunosorbent assay
EPA	Environmental Protection Agency (USA)
ETU	ethylene thiourea
EU	ethylene urea
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act (US legislation)
FT_4	free thyroxine
GC	Gas chromatography
GC/MS	gas chromatography with mass spectroscopy
GLP	Good Laboratory Practice
g	gram(s)
G-GT	gamma glutamic transferase
GI	gastro-intestinal tract
GLP	good laboratory practice
GSD	gravimetric standard deviation
GSH	glutathione
GST	glutathione-S-tranferase
Hb	haemoglobin
h	hour(s)
^3H	tritium, radioactive isotope
ha	hectare
HPLC	High performance liquid chromatography
HSE	Health and Safety Executive
I	iodine
Ig	immunoglobulin
IL	interleukin
i.p.	intraperitoneal
IR	Infra-red spectroscopy
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
K_d	soil/water distribution ratio
K_{om}	adsorption coefficient with respect to the organic matter content
KCl	potassium chloride
l	litre(s)
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LOAEL	lowest observed adverse effect level
LOEC	lowest observed effect concentration
LSC	liquid scintillation counting
m	metre(s)
M	molar
MATC	maximum acceptable toxicant concentration
meq	milliequivalents
MFO	mixed function oxidase
mg	milligrams
MIC	minimum inhibitory concentration
min	minute(s)
ml	millilitre(s)
mm	millimetres

mmol	millimole(s)
N	normal
N-AcEDA	N-acetylethylenediamine
NADPH	nicotine adenine dinucleotide phosphate
NaOH	sodium hydroxide
NCE	normochromatic erythrocytes
NMR	Nuclear magnetic resonance
NOEC	no observable effect concentration
NOEL	no observable effect level
NOAEL	no observable adverse effect level
NTP	National Testing Programme (USA)
ODC	ornithine decarboxylase
OECD	Organisation for Economic Co-operation and Development
Pa	Pascal(s)
P_{ow}	Octanol/water partition coefficient
pADPRP	poly(ADP-ribose)polymerase
PB	protein bound
PBMC	peripheral blood mononuclear cells
PBL	peripheral blood lymphocytes
PCE	polychromatic erythrocytes
PCV	packed cell volume
PEC	predicted environmental concentrations
PEC_{water}	predicted environmental concentrations in water
$PEC_{sediment}$	predicted environmental concentrations in sediment
PNA	para-nitrophenyl acetate
PNEC	predicted no-effect concentration
ppb	parts per billion
PPE	personal protective equipment
ppm	parts per million
PVC	Polyvinyl chloride
QA	quality assurance
QWASI	quantitative water air sediment interface
REMA	Regulatory Environmental Modelling of Antifoulants
RNA	ribonucleic acid
rpm	revolutions per minute
s	second
SCE	sister chromatid exchange
sd	standard deviation
SDS	sodium dodecyl sulphate
SLS	sodium lauryl sulphate
SPC	Self polishing co-polymer
SPE	solid phase extraction
STP	sewage treatment processes
$t_{½}$	half life
T_3	triiodothyronine
T_4	thyroxine
TBT	tributyltin
TBTO	tributyltin oxide
TER	toxicity:exposure ratio
TFT	trifluorothymidine

TLC	thin-layer chromatography
TSH	thyroid stimulating hormone
TU	thiourea
TWA	time weighted average
UDS	unscheduled DNA synthesis
UK	United Kingdom
US	United States
USDA	United States Department of Agriculture
UV	ultra-violet
µg	microgram(s)
µl	microlitre(s)
µm	micrometre(s)
µmol	micromole(s)
US EPA	United States Environmental Protection Agency
UV	Ultra violet
W	watts
w/w	weight per unit weight
w/v	weight per unit volume
$ZnCl_2$	zinc chloride

OVERALL SUMMARY

Introduction

Zineb is one of a group of compounds known as the ethylene bis-dithiocarbamates (EBDCs). It is used as a fungicide in agriculture and is also used in approved antifouling products. Following the reviews of triorganotins and copper compounds in antifouling products, high priority has been given to reviews of all the 'booster biocides', the organic active ingredients used in association with copper and/or triorganotins. The ACP agreed a timetable to complete the hazard evaluations of these substances by 2000 so all of the reviews could be considered together and a concerted approach to the environmental risk assessment undertaken. Committee Members examined overviews of the available mammalian toxicology in May 1997 and subsequently required that those booster biocides that might be of particular concern for human health should be reviewed urgently. Zineb was one of these substances and the ACP considered an evaluation of the physicochemical and mammalian toxicology data submitted to the zineb review in October 1999. These data included studies on the related compound mancozeb. The ACP accepted the manufacturer's argument that the toxicological data could be used to 'read-across' to zineb. Additional PPE was specified for professional users and data on skin penetration and some physical chemistry endpoints were required. These requirements had been conveyed to approval holders but the deadline for data submission had not yet expired at the time that the ACP considered this review.

This document includes the evaluation of physicochemical and mammalian toxicology data on zineb that were considered in 1999. The assessment of risk to users and bystanders was revised, as requested by the ACP, to take into account all factors affecting exposure and increase the clarity and transparency. The document also includes an evaluation of the available environmental and efficacy data on zineb. The environmental data on each of the booster biocides have previously been considered by the Environmental Panel of the ACP, together with an assessment of the risks to the environment. The environmental risk assessment for each of the booster biocides has been presented in one overall document entitled "Environmental risk Assessment of Booster Biocides inAntifouling Products' (ACP 2002) and takes account of the views of the Environmental Panel. This is summarised in Section 7.

Physicochemical properties

Zinc ethylenebis (dithiocarbamate) (polymeric) (IUPAC) has a purity of 92 - 95 % w/w or 94 - 97 % w/w. It is an off - white fine powder of molecular mass (275.8); relative density 1.96 at 20 ^{o}C; estimated vapour pressure < 1.11 x 10^{-2} Pa (at 25 ^{o}C); zineb decomposes before melting (157 ^{o}C); it is sparingly soluble in water (0.07 - 10 mg l^{-1} at 20 ^{o}C) with log P_{ow} 0.77 (at 25 ^{o}C). Analytical methods have been provided for determination of both the active ingredient and inert ingredients in technical zineb; and for the determination of zineb in formulations and water.

Toxicokinetics

No useful data is available on the toxicokinetics of zineb in humans. There is a variable amount of information available from studies in experimental animals, depending upon the route of exposure. Furthermore, of those studies that are available, none have investigated the fate of the zinc ion and all have focused on the toxicokinetics of the organic portion of the

molecule. Although the fate of the zinc from the molecule is unclear, the toxicology profile of zineb does not appear to indicate any significant involvement of zinc in its toxicity.

Oral

The toxicokinetics of the organic part of zineb following oral dosing has been well studied in rats, though over a relatively small dose range, with limited studies in the mouse and marmoset. Following gavage dosing (5 or 50 mg kg^{-1}) in the rat around 50-70 % of the administered dose is taken up rapidly across the GI tract. An older dietary study in rats has suggested a lower (up to 20 %) uptake but these data should be viewed with some caution because of limitations in the design and reporting of the study. Oral gavage dosing of marmosets has also indicated that at least 50-60 % of the administered dose is taken up rapidly in this species. The only study in the mouse indicated uptake to be low at ~6 % but as recovery of the administered dose was significantly incomplete this value should be treated with caution. Overall, uptake following oral dosing in the rat and marmoset is judged to be moderate and rapid.

Distribution data are only available for the rat. Once absorbed, in rats the organic portion of the parent molecule and/or its metabolites is widely distributed throughout the body, with the muscle, liver, kidney, adrenal and thyroid all being particular sites of uptake (in terms of tissue concentration the thyroid is the most significant organ). With respect to metabolism, the available information indicates that in all species studied zineb is metabolised to ETU. The most extensive studies are for the rat, in which it has been shown that at least 20 % of an administered oral dose is converted to ETU, with the presence of the ETU metabolites EU and TU found in significant quantities (4-15 % of an administered dose). EDA (~10 % of a dose) has also been found to be produced from zineb, as has CS_2 qualitatively. Studies in the marmoset indicate that at least 20 % of an administered dose is converted to ETU in this species. It is difficult to define a value for the mouse, as recovery was incomplete.

A proposed metabolic pathway for zineb following oral dosing is shown at Figure 3.1. Following oral dosing, the organic portion of zineb is excreted relatively rapidly in all species studied, mainly via the urine and in the faeces. Urinary excretion is largely in the form of metabolites. Faecal content is mainly unabsorbed zineb in rats since biliary excretion accounts for only a small amount (1-6 %) of an administered dose. Small amounts, a few percent at most, of an administered dose have been found to be excreted in expired air as CO_2. Only relatively small amounts (1-2 %) have been found to be retained in the body in the rat and marmoset (incomplete recovery in the mouse makes it difficult to judge retention) following a single oral dose. There is some limited evidence from the rat for some bioaccumulation following repeated daily dosing, although the toxicokinetics otherwise were essentially similar as those for single dosing.

A similar oral toxicokinetics database is available for the organic portion of the structurally related mancozeb with extensive studies in the rat and mouse and some limited information from the dog and lactating goat. No information was available on the fate of the zinc or manganese though as for zineb toxicodynamic data suggest that these do not play a significant role in toxicity. In general the toxicokinetics of mancozeb following oral dosing is broadly similar to that of zineb. Absorption across the GI tract appears to be similar with around 50-60 % of the organic portion being taken up rapidly in rats. Slightly less (up to 45 %) but rapid uptake was seen in the mouse and, based on limited information, around 50 % in lactating goats.

Distribution of the absorbed portion of mancozeb and/or its metabolites is largely similar to that of zineb in that it is widespread, with the muscle, thyroid and liver being particular tissues for uptake in the rat. In the mouse, the evidence available did not indicate that uptake into the thyroid was more significant than other tissues. Studies in the goat indicated that some (possibly up to half) of the available carbon is recruited into general body pools.

The metabolic profile of mancozeb is also similar to that of zineb in the rat with ETU again being a significant metabolite accounting for the conversion of at least 20 % of an administered dose. In contrast, ETU would appear to be a relatively minor metabolite of mancozeb in the mouse. A proposed metabolic pathway for mancozeb following oral dosing is shown at Figure 3.2. The excretion of mancozeb, like zineb, is rapid and principally via the faeces and urine, with small amounts excreted in expired air as CO_2 and CS_2. Evidence from the lactating goat indicates that a small proportion of the organic portion of mancozeb and/or its metabolites is excreted via the breast milk. Given many of the similarities of the toxicokinetic properties of zineb and mancozeb, it would seem reasonable to conclude that a small amount of zineb and/or its metabolites also be available to breast milk.

Dermal

The information available for the toxicokinetics of zineb following dermal dosing is limited to one good quality study in the rat. This indicated that over a wide dose range covering three orders of magnitude (expressed relative to body weight or surface area) that relatively little of the applied dose (<1 %) was absorbed into the body. Although of poor quality, a dermal study in rats with mancozeb generally confirmed the low bioavailability via this route. Mancozeb and ETU were detected in the blood of rabbits repeatedly exposed to mancozeb for 21 d. However, it is not possible to quantitate the extent of uptake directly from this study.

Inhalation

There are no specific studies on the toxicokinetics of zineb following inhalation exposure. Qualitative information from a limited repeat inhalation exposure toxicity study in rats with solid zineb powder suggests that uptake and distribution of the substance and/or its metabolites may occur from the respiratory tract as indicated by toxicity in the liver and kidneys. This view is supported by the detection of EBDC and ETU in the blood, urine and thyroid of rats repeatedly exposed by inhalation to the structurally related mancozeb. The presence of a typical response to the presence of a dust in the respiratory tract of the zineb-exposed rats suggests that uptake may be limited via this route. Overall, though, the data available do not allow any quantitative conclusions to be drawn with respect to the toxicokinetics of zineb following inhalation exposure.

Placental transfer

No specific information is available on whether or not zineb can cross the placenta and be taken up by developing offspring. It is known that the metabolite ETU ***(outlined in Section 3.3.7.1.3 of the main document)*** *is able to reach the offspring as evidenced by effects on development. It would therefore be reasonable to assume that ETU generated following the metabolism of zineb may also be able to reach the developing offspring.*

Overall, on considering the data available on the relative toxicokinetics of the organic portions of zineb and mancozeb, it would appear that on the whole the two substances are handled, at least in the rat, in a generally similar manner (both qualitatively and quantitatively). It would therefore appear reasonable to use toxicity data for mancozeb to read across to zineb where it is believed that any effects seen reflect a similar metabolite-related aetiology. Although extensive information was available from studies in rats and limited data from other species, it was not possible to say whether or not the toxicokinetics of zineb or mancozeb seen in studies in animals following oral, dermal or inhalation routes of exposure would be similar in humans.

Mammalian Toxicology

Acute toxicity

No useful human data are available. There are few good quality animal studies available which have investigated the acute toxicity of zineb. However, those that are available present a reasonably consistent picture. Zineb is of low acute toxicity by the oral route, with LD_{50} values in excess of the highest doses tested that are of the order of grams per kilogram. Mancozeb, a structurally similar substance is also of low acute oral toxicity. From the limited information available, the acute toxicity of zineb via the dermal route appears to be low, which is consistent with the low oral toxicity and poor bioavailability via this route. The only available study on the acute inhalation toxicity of zineb is of too poor a quality to draw any conclusions. An inhalation study on mancozeb resulted in the expression of acute effects with deaths in male rats and signs of toxicity in both sexes at around 5 mg l^{-1} suggesting low acute inhalation toxicity. This appeared to be related to local effects in the respiratory tract.

There are few reports available on the skin irritation potential of zineb. Those that are available are old and have limitations in the reporting. From the few data available (two animal and one human study) the indications are that zineb is unlikely to possess any significant skin irritation potential. This view is supported by the data for the structurally related mancozeb. The data for eye irritation potential are equally as poor but suggest little eye irritation potential for zineb. In contrast a study conducted to modern standards with good reporting indicates that mancozeb has moderate eye irritation potential, raising questions about the adequacy of the eye irritation data available for zineb. No data were available on the potential for zineb to act as a respiratory tract irritant. Exposure concentrations of mancozeb producing lethal effects were found to induce histopathological damage to the larynx. However, it is unclear if such effects occurred at lower non-lethal exposure concentrations. Overall, no conclusions on the respiratory tract irritation potential of zineb can be drawn.

A number of human case reports are available in which individuals have presented with contact dermatitis and subsequently been found to respond to skin challenge with zineb. However, none of these cases provides conclusive evidence that zineb itself possess skin sensitization potential. Zineb was found to produce positive results in a limited maximisation test in guinea pigs. The structurally related mancozeb also produced positive results in the same guinea pig study and was also shown to be a skin sensitizer in a more recent Buehler study. Overall, although the database is somewhat limited, taken altogether the available information indicates that on balance zineb is likely to possess skin sensitizing potential. No data are available on whether or not zineb is likely to be a respiratory sensitizer.

Repeat dose toxicity

Sub-chronic studies (≤ 90 days duration)

Relatively few data are available on the effects of zineb following sub-chronic exposure in animals. Some information is available from 28 d studies in rats and 90 d studies in rabbits conducted via the oral route, but none via the inhalation or dermal routes. More data are available (essentially 90 d studies) for the structurally related mancozeb and the principal metabolite of both zineb and mancozeb, ETU. Qualitatively, where comparable, the toxicity profile of all three substances is similar, with the key common finding being effects on the thyroid and liver MFO activity. In addition, mancozeb was found to induce some effects not seen with zineb but this may be due to the more thorough and extended nature of those studies available. Because the composition of the tested zineb and mancozeb is often unclear, it is difficult to say whether the effects seen are due to the parent compound, its metabolites, contamination with other substances (particularly ETU), or a combination of these. However, on balance it would seem reasonable to conclude that at least the effects seen on the thyroid and liver MFO activity induced by zineb and mancozeb are likely to be a consequence of either production of ETU by metabolism and/or contamination of the test material with ETU. Given this probable common aetiology for these effects, it would seem reasonable to compare the toxicological properties of zineb with those of mancozeb.

In both rat and rabbit treated orally with zineb, inhibition of liver MFO and hypothyroidism were observed. A NOAEL for thyroid effects could not be established (although low doses of up to 500 ppb for 5 d were without effect), with the lowest doses tested, 50 mg kg^{-1} d^{-1} in the rat and 90 mg kg^{-1} d^{-1} in the rabbit, producing effects on the thyroid. These effects ranged from increased thyroid weight at the lowest doses, to decreased T_4 and thyroid follicular cell hypertrophy at the higher doses (rat, 1000 mg kg^{-1} d^{-1}; rabbit, 180 mg kg^{-1} d^{-1}). Regarding inhibition of hepatic MFO activity, the NOEL for this effect was determined in rats to be 60 ppm in the diet (~6 mg kg^{-1} d^{-1}). The relevance of the changes in MFO activity are unclear, but inhibition of the ability of the liver to metabolise may be of concern in relation to the clearance of xenobiotic substances from the body.

The structurally related mancozeb has been studied in rats and dogs via oral dosing. Qualitatively similar findings to those with zineb in the thyroid and liver were observed but because of the much better quality of the studies available more useful quantitative information can be derived. In rats, the range of NOAELs for these effects was 7-10 mg kg^{-1} d^{-1}. Effects on thyroid (increased weight, decreases in T_4, increases in TSH, thyroid cell hypertrophy and hyperplasia) showed an increasing severity with dose, from dose levels equivalent to 15 mg kg^{-1} d^{-1}. A reduction in liver MFO activity was observed in one study at high dose levels, with no effects seen at 250 ppm (~15-18 mg kg^{-1} d^{-1}). In addition to effects on the thyroid and liver, lesions (demyelination, Schwann cell proliferation and neurofibrillary degeneration) on the long nerves (sciatic, tibial and sural nerves) were observed in rats at ~50 mg kg^{-1} d^{-1} and above. Muscular atrophy of the hindquarters was also observed at higher (340 mg kg^{-1} d^{-1} and above) doses. Based on studies performed in rats, which included particular investigation of nervous tissue, the NOAEL for these effects was around 8-10 mg kg^{-1} d^{-1}, similar to those for effects on the thyroid and liver. In the absence of similar investigations in other species and information on the underlying mechanism, the relevance to human health of these neuropathological findings seen in rats exposed to mancozeb is uncertain, but cannot be discounted as being of no relevance.

In dogs administered mancozeb in capsules or in the diet a similar picture of thyroid effects to that seen in the rat was observed. In addition, hepatotoxicity, haematological effects (reductions in PCV, haemoglobin concentration and erythrocyte count) and effects on the testes and ovary were seen in this species. In the dietary study a clear NOAEL of 100 ppm (3 mg kg^{-1} d^{-1}) was established but a NOAEL was not established in the capsule study which used dose levels down to 6 mg kg^{-1} d^{-1}. Haematological effects were observed at dose levels of ~30 mg kg^{-1} d^{-1} and above. Effects on the testes, manifest by reduced spermatogenesis, were observed at 34 mg kg^{-1} d^{-1} and above. These effects may have either a direct effect of mancozeb on the testes or have been mediated via effects on thyroid function. Thyroid effects were seen in both studies, with histopathological changes seen at all dose levels (~from 6 mg kg^{-1} d^{-1}) in the capsule study. Given the clear NOAEL of 3 mg kg^{-1} d^{-1} in the dietary study, this may be around a threshold for these effects; however, there is also the potential for differences in toxicokinetics, and hence toxicodynamics, following dietary as opposed to bolus dosing. At the highest doses tested in the dog (102-340 mg kg^{-1} d^{-1}) signs of severe toxicity were observed with effects seen in the liver and thymus (reduced weight and lymphoid depletion of the cortex).

Toxicokinetic data indicate that ETU is formed as a primary metabolite of zineb and mancozeb. It is probable that the effects on the thyroid, at least, are related to the presence of ETU either from metabolism and/or as a contaminant of the tested materials. The toxicological effects of ETU administration have been studied in rats, mice and dogs. A consistent picture of thyroid target organ toxicity was found, with other effects probably related to malnutrition also observed. In the rat, a NOAEL could not be identified due to the presence of thyroid follicular cell hyperplasia at 3-4 mg kg^{-1} d^{-1}, the lowest doses tested. In mice a NOAEL of 30 mg kg^{-1} d^{-1} was identified with thyroid follicular cell hyperplasia apparent at $\geq$ 60 mg kg^{-1} d^{-1}. In dogs, a NOAEL of 0.4 mg kg^{-1} d^{-1} for ETU was identified with marginal effects (mainly relating to anaemia, also seen with mancozeb) at ~6 mg kg^{-1} d^{-1} and clear signs of effects on the thyroid seen at doses around 10-fold higher. In general, therefore, the toxic effects of ETU are essentially the same qualitatively as those seen with zineb and mancozeb. However, there are quantitative differences that are probably reflective of the difference in toxicokinetics between ETU administered directly and being produced as a metabolite of another compound. Overall though, from the information available it would seem reasonable to conclude that the effects produced by zineb and mancozeb, at least on the thyroid and possibly on haematopoesis in the dog, have a common origin via ETU. The relationship between zineb, mancozeb and ETU with respect to thyroid toxicity is discussed further in the summary of carcinogenicity.

Regarding the dermal route of exposure, no studies have been performed using zineb. Toxicokinetic data ***(outlined in Section 3.2.1.2.1)*** *indicate that there is a relatively low potential for dermal absorption of zineb and it would be predicted to be of low sub-chronic toxicity when applied directly to the skin. Consistent with this picture, conventional studies of mancozeb applied to the skin of rats and rabbits for 28 days showed reduced body weight gain as the only effect seen only in rabbits at a level of 1000 mg kg^{-1} d^{-1}, well in excess of the dose levels associated with systemic toxicity following oral dosing. No effects were seen in the rat at 1000 mg kg^{-1} d^{-1} or in the rabbit at 250 mg kg^{-1} d^{-1}.*

No sub-chronic studies of repeated inhalation exposure to zineb are available. In the case of mancozeb rats exposed to 127 mg.m^{-3}, 6 h d^{-1} (estimated to be equivalent to 30.5 mg kg^{-1} d^{-1}) 5 days per week for 90 days showed a decrease in T_4 levels and an increase in TSH

indicating a potential for absorption, metabolism and toxicity via this route of exposure. No effects were seen at 40 mg m^{-3} (estimated to be equivalent to 9.6 mg kg^{-1} d^{-1}).

Chronic studies (>90 days duration)

As for sub-chronic toxicity, the data available on the toxicity of zineb following chronic dosing are limited to relatively few studies performed in rats and dogs mainly via the oral route. More information, generally of a better quality, is available for the structurally related mancozeb and the principal metabolite ETU. The toxicological profile that emerges following chronic dosing is essentially similar to that following shorter term repeat dosing with all three substances and thus comparison between them is again considered to be a reasonable approach to adopt.

Chronic oral dosing studies have been carried out on zineb in rats and dogs. These have demonstrated the thyroid to be a principal target tissue with effects also seen in the kidney at relatively high (600 mg kg^{-1} d^{-1}) doses. The studies in rats did not generally result in a robust NOAEL, with effects on the thyroid (weight increase and histopathological changes including cellular hypertrophy and hyperplasia) being seen at all doses tested (from ~75 mg kg^{-1} d^{-1}). Essentially no effects were seen in one study at 9.6 mg kg^{-1} d^{-1} administered to rats twice weekly for 4.5 months. However this value should be treated with some caution because of the limitations of the dosing regimen and uncertainties over some of the experimental techniques employed. No effects were seen at 2000 ppm zineb in the diet (~50 mg kg^{-1} d^{-1}) in an older study in dogs with effects on the thyroid seen at 10000 ppm (~250 mg kg^{-1} d^{-1}).

The chronic toxicity of mancozeb with oral exposure, revealing a similar pattern of effects to those of zineb, has been studied in rats, mice and dogs, often as part of cancer bioassays. In general, all the studies are of a much better quality compared to those available for zineb. In rats, NOAELs were identified in the range 4 - 7 mg kg^{-1} d^{-1}, with signs of effects in the thyroid being seen at dose levels of ~15 mg kg^{-1} d^{-1} and above. Unfortunately, it is difficult to make direct quantitative comparisons with zineb because of the difference in the quality of the studies available and the generally higher doses of zineb used. However, from the limited information available, it would broadly appear that the same type (thyroid) and severity of effects are seen over a similar dose range for the two substances. Thus it is probably reasonable to use the mancozeb rat NOAELs as predictions of NOAELs for zineb. In mice, a NOAEL of 13-18 mg kg^{-1} d^{-1} was identified in a 78 week study with mancozeb with effects seen on the thyroid at ~130 mg kg^{-1} d^{-1}, the highest dose tested.

As there are no studies in mice using zineb or from shorter-term exposure with mancozeb, few comparisons can be made, although mice appear to be somewhat less responsive than rats to mancozeb (and thus probably zineb). In one (52 week) of the two dog studies available, a NOAEL of ~8 mg kg^{-1} d^{-1} for mancozeb was identified. Evidence of slight reductions in haemoglobin, red blood cell count and packed cell volume were seen at ~30 mg kg^{-1} d^{-1} and above, with effects on the thyroid (follicular distension, increased absolute weight and colloid retention) and liver (Kupffer cell pigment deposits) only seen at ~50 mg kg^{-1} d^{-1}. Comparison of these studies with the one available for zineb suggests that zineb may be less active in the dog than mancozeb, though less reliance can be placed on the older, less well-reported zineb study.

Chronic oral dosing with ETU in the rat was found to produce effects on the thyroid (increased weight, follicular and nodular cell hyperplasia) at ~10 mg kg^{-1} d^{-1}, with no effects

seen at ~0.4 mg kg^{-1} d^{-1}. In dogs no effects were seen at ~0.2 mg kg^{-1} d^{-1}. Effects on the liver (Kupffer cell pigment deposits) and thyroid (increased weight, follicular cell hypertrophy with colloid retention) were seen at ~2 mg kg^{-1} d^{-1} with increased severity at ~20 mg kg^{-1} d^{-1}.

Overall, the effects of chronic oral dosing with zineb, mancozeb and ETU are qualitatively similar to those seen after shorter term dosing, with effects on the thyroid and liver most prominent. The effects on the thyroid induced by zineb and mancozeb are consistent with those known to be produced by ETU as exemplified by the submitted chronic studies on ETU in rats and dogs. It is difficult to make direct comparisons on a quantitative basis between zineb and mancozeb with the relative qualities of the databases available. However, taking into account the similarities in toxicokinetics between the substances, it would seem reasonable to use the information available for mancozeb where data are limited for zineb, particularly where a common aetiology for effects is likely to exist. Such a read across would suggest NOAELs for zineb for oral dosing of around 4-10 mg kg^{-1} d^{-1} for the rat and dog, with a slightly higher value for the mouse. In contrast, ETU appears to be more potent than mancozeb (and thus probably zineb) with NOAELs and LOAELs up to around 10-fold lower. This difference, though, is perhaps to be expected on the basis that comparison is being made between studies involving the direct application of ETU with those where ETU is either generated principally as a metabolite and/or may be present as a low level contaminant. What is critical is that the effects seen are basically similar across all three substances, indicating that ETU is probably the main factor in the induction of effects, at least on the thyroid, by zineb and mancozeb.

Chronic dosing via other routes of exposure has been poorly studied. No dermal dosing studies are available for zineb or ETU and no useful conclusions can be drawn from the only study available for mancozeb. However, given the low bioavailability of zineb via this route, it would be predicted to be of low chronic dose toxicity when administered to the skin (increasing the uptake via the skin would undoubtedly alter this profile).

With respect to inhalation exposure, the only study available on zineb indicates that the liver and kidney may be target tissues. However, since the thyroid was not studied and there were limitations in the reporting of the study it is difficult to draw any firm quantitative conclusions. No useful studies were available on mancozeb or ETU following inhalation exposure.

Human data (repeated exposures)

No studies of workers exposed only to zineb or mancozeb are available.

In groups of flower bulb workers, slight differences were seen in measurements of nerve conduction velocities, however, the clinical significance of these findings is uncertain. It is not possible to draw any conclusions on the role zineb may have played.

In one study of workers exposed to a variety of pesticides, typically including mancozeb, a proportion of crop sprayers showed a slightly greater blood level of ETU compared to lesser exposed farm owners and non-exposed controls. No effects on serum levels of TSH and T4 were detected in the potentially exposed workers. Supporting data are available from a study of workers involved in the production of mancozeb, where ETU-haemoglobin adducts were found in 40 % of workers. No other information on ETU-adducts following exposure to

EBDCs is available. However, this finding is consistent with the observation from studies in animals of the metabolism of EBDCs to ETU.

Workers exposed to mancozeb and a variety of other chemicals including CS_2 showed a statistically significant increase in lymphocyte proliferative response and IL2 production. Due to inadequacies in exposure assessment in regard of other chemicals and previous exposures, and in light of the fact that no clinical signs of immunologically mediated disease was detected, the toxicological significance of this finding is unclear.

Genotoxicity

With respect to genotoxicity, cytogenetic analysis of the peripheral lymphocytes of workers potentially exposed to zineb and/or mancozeb have been carried out ***(outlined in Section 3.3.5.2.3)****. However, due to limitations in the design and reporting of the studies no firm conclusions could be drawn from them. Zineb has been tested for its mutagenic potential in a variety of in vitro and in vivo assays and in rodent somatic cell in vivo assays. The balance of data available clearly indicates a lack of genotoxic activity for zineb in vitro in either bacteria or mammalian somatic cells and in vivo in somatic cells following oral dosing. This profile of a lack of mutagenic potential is supported by the negative results in equivalent studies for both the structurally-related mancozeb and the common metabolite ETU. No studies in germ cells are available. However, given the lack of genotoxic activity in somatic cells both in vitro and in vivo there is no basis for any concern that zineb could be genotoxic to the germ cells.*

Carcinogenicity

There are no carcinogenicity studies available of an adequate quality or standard investigating the carcinogenic potential of zineb via any route of exposure. Consequently, no conclusions regarding the carcinogenic potential of zineb can be made based directly on the available zineb data. However, based on similarities in toxicokinetics and toxicodynamics it is predicted that zineb would show a similar, qualitative and quantitative carcinogenicity profile to the structurally related mancozeb. Furthermore, studies are also available on the principal metabolite of both zineb and mancozeb, ETU. There are no inhalation or adequate dermal carcinogenicity studies available for mancozeb. Based on toxicokinetic information it is possible to predict that the carcinogenic potential of zineb (and mancozeb) in rodents via inhalation, will be similar to that observed via oral administration. However, it is not possible to quantify the levels at which a response would occur. Toxicokinetic data also demonstrate negligible dermal absorption of both zineb and mancozeb (<1 %) and therefore it can be predicted that neither zineb nor mancozeb are likely to be carcinogenic via the dermal route of exposure.

Two well-conducted oral studies in rats with mancozeb are available, the results of which indicate that mancozeb induces thyroid gland follicular cell adenomas and carcinomas in rats following 24-month dietary administration at concentration of 750 ppm (equivalent to 31-40 mg kg^{-1} d^{-1}). No tumours were observed in mice, in a well-conducted oral study with mancozeb at doses of up to 1000 ppm (equivalent to 13-18 mg kg^{-1} d^{-1}). This latter observation is of some significance since ETU was found to induce both thyroid and liver tumours in mice at doses of 1000 ppm and above. Toxicokinetic data indicate that in the mouse ETU is a minor metabolite following oral administration. Therefore, it is likely that the levels of ETU available to the mouse via metabolism of mancozeb in this study were not

sufficiently high to produce any effects. It is probable that at doses high enough in mice to result in the production of significant levels of ETU then carcinogenic effects might occur. However, from the data available this would seem to be at dose levels significantly above those seen to produce effects in the more sensitive species, the rat. Since the thyroid was the key target tissue for both non-neoplastic and neoplastic changes in the rat, this is considered to be the principal tissue of concern with respect to carcinogenic activity of mancozeb, and thus by analogy, zineb.

In view of the lack of genotoxic activity of mancozeb (and zineb), it is concluded that the thyroid tumours seen in rats arose as a result of the chronic hyperplastic response of the thyroid follicular cells, induced by a direct action of mancozeb or more probably its metabolite ETU. ETU was found to induce thyroid tumours in rats and mice in the submitted studies. This substance is well known and documented to be capable of inducing thyroid tumours in rodents. It is believed that ETU exerts this effect through a non-genotoxic mechanism involving the disruption of thyroid hormone homeostasis by inhibition of thyroid peroxidase leading to a reduction in the production of T_4 and hence a reduction in circulating levels of T_4. This in turn leads to an increase in trophic stimulation (increased secretion of TSH by the pituitary) of the thyroid. This may lead to a hypertrophic and hyperplastic response in the case of long-term thyroxine deficit and continued trophic stimulation. The hyperplastic state is believed to lead to an increased potential for neoplastic change and tumour development. Such a mechanism is considered to be of possible relevance to human health, though rodents, particularly the rat, may be more responsive because of the inherently normal higher activity of the thyroid. Furthermore, because of the presence of thyroid binding globulin in humans, which can act as a reserve for thyroid hormone, any extrapolation of data from rat to human is rendered more uncertain. The effects which signal this proposed sequence of events (decreased T_4, increased TSH, thyroid follicular cell hypertrophy and hyperplasia) are seen as a consequence of exposure to zineb and more clearly for mancozeb. In reviewing all the information available these changes can be seen to be related both to the dose of substance administered and the duration of the exposure. It is likely that the effects seen with zineb and mancozeb are due to the presence of ETU in the test animals, most probably as a consequence of metabolism. There is also the possibility of an additional contribution of low level ETU contamination in the test material. Whatever the actual contribution from these potential sources, it is probable that the long-term exposure of the test animals to ETU at a high enough dose level of the parent compound leads to an inhibition of thyroid hormone production and ultimately to tumour formation via the mechanism described. Since, from the limited toxicity data available and taking into account the toxicokinetic information, zineb is toxicologically similar to mancozeb it is predicted that at high enough doses zineb would induce similar neoplastic changes, including the induction of tumours, in the thyroid. Since the mechanism outlined above is likely to have a threshold (i.e. the underlying inhibition of thyroid peroxidase) then a long term NOAEL for the effects on the thyroid should also be, in principle, a NOAEL for potential neoplastic change and tumour formation. For mancozeb, the lowest NOAEL for chronic oral administration in the most sensitive species, the rat, is ~5 mg kg^{-1} d^{-1}. It is likely that a similar value would be appropriate for zineb. In relation to the liver tumours produced in mice by ETU, as indicated above, studies on mancozeb resulted in no effects in the liver of mice at doses of up to 13-18 mg kg^{-1} d^{-1}. This is clearly above the NOAEL for thyroid effects and thus if the liver was a potential target tissue for zineb in the mouse this would be at dose levels likely to be already associated with concerns for thyroid carcinogenicity.

Reproductive toxicity

Only limited information on the developmental effects of zineb is available and such data are essentially restricted to effects in rats using the oral route. Marked developmental effects (micrognathia, phocomelia, encephalocoele and hydrocephalus) were seen in the pups of dams that received 2000 mg kg^{-1} orally during gestation. However, it was unclear whether or not there were also signs of maternal toxicity at this exposure level. No developmental effects were seen in rats at 1000 mg kg^{-1} in the same study. One inhalation study is available but due to limitations in reporting no useful conclusions can be drawn. Information from other species or routes of administration is lacking. More information is available on the structurally related mancozeb. Oral dosing with mancozeb to rats during gestation resulted in no effects on development at doses up to 60 mg kg^{-1} d^{-1}. An increase, compared to controls, in skeletal anomalies (reduced ossification of the intraparietal bone and increase in the size of the anterior fontanelle) were observed at 360 mg kg^{-1} d^{-1}, the highest dose used, a level associated with marked maternal toxicity. A brief report of a study in rabbits suggests no effects on development at 30 mg kg^{-1} d^{-1} with severe maternal toxicity occurring at 80 mg kg^{-1} d^{-1}, the next dose level tested. Mancozeb has also been tested via inhalation exposure with no effects seen on development at 55 mg m^{-3}, a level associated with maternal toxicity. An increase in skeletal anomalies was seen in fetuses at 110 mg m^{-3}. Overall, there is some evidence that mancozeb may induce an increase in skeletal anomalies, although only at dose levels associated with maternal toxicity. ETU, the common metabolite of zineb and mancozeb is recognised as possessing the ability to affect development. In the rat, developmental effects (particularly hydrocephaly, and skeletal malformations) have been demonstrated in the absence of maternal toxicity following a single oral administration of 30 mg kg^{-1} at any time during days 13-20 of gestation, or repeated doses of 5 mg kg^{-1} during days 6-15. At higher exposures (around 200 mg kg^{-1}) mesenchymal necrosis of forelimb buds was noted, still in the absence of maternal toxicity.

There are no conventional studies that have specifically investigated effects on fertility. In the absence of high quality data on zineb, it is possible to read across information from the structurally-related EBDC, mancozeb, and one of the potential active metabolites, ETU. Data only via the oral route of administration are available.

A high quality multi-generation study using the oral gavage route indicates that there were no adverse effects on fertility even at doses that resulted in parental toxicity (about 80 mg kg^{-1} d^{-1} repeatedly). A similar study indicated that ETU itself did not result in impaired fertility at doses up to around 20 mg kg^{-1} d^{-1}. Hence, overall, although the fertility data on zineb are limited, the overall conclusion from the database including mancozeb and ETU is that such effects would not be anticipated at quantitatively similar doses.

Human Data

The information from studies in humans has been covered in detail under each toxicological endpoint. The following briefly summarises the available information and indicates those Sections where more details are presented.

Only a single case report was available relating to acute toxicity in a 42 year old male who used a zineb-mancozeb mixture to spray cucumbers ***(outlined in Section 3.3.1.1.4)****. Although toxic signs and symptoms were reported, probably due to his exposure, no firm conclusions can be drawn given a lack of objective information on exposure conditions. No skin irritation*

*was observed in 49/50 volunteers exposed to a '65 % zineb commercial wettable powder' **(outlined in Section 3.3.2.2)**. No information is available on eye or respiratory tract irritation in humans. With respect to skin sensitization, a number of human case reports are available in which individuals have presented with **(outlined in Section 3.3.3.1.2)** contact dermatitis and subsequently been found to respond to skin challenge with zineb. However, none of the cases provides conclusive evidence that zineb itself possess skin sensitization potential. No data are available for humans on the respiratory sensitization potential of zineb.*

*No studies of workers repeatedly exposed only to zineb or mancozeb are available. In groups of flower bulb workers, slight differences were seen in measurements of nerve conduction velocities, however, the clinical significance of these findings is uncertain. It is not possible to draw any conclusions on the role zineb may have played **(outlined in Section 3.3.4.5)**.*

*In one study of workers exposed to a variety of pesticides, typically including mancozeb, a proportion of crop sprayers showed a slightly greater blood level of ETU compared to lesser exposed farm owners and non-exposed controls **(outlined in Section 3.3.4.5)**. No effects on serum levels of TSH and T_4 were detected in the potentially exposed workers. Supporting data are available from a study of workers involved in the production of mancozeb, where ETU-haemoglobin adducts were found in 40% of workers. No other information on ETU-adducts following exposure to EBDCs is available. However, this finding is consistent with the observation from studies in animals of the metabolism of EBDCs to ETU.*

*Workers exposed to mancozeb and a variety of other chemicals including CS_2 showed a statistically significant increase in lymphocyte proliferative response and IL2 production **(outlined in Section 3.3.4.5)**. Due to inadequacies in exposure assessment in regard of other chemicals and previous exposures, and in light of the fact that no clinical signs of immunologically mediated disease was detected, the toxicological significance of this finding is unclear.*

*With respect to genotoxicity, cytogenetic analysis of the peripheral lymphocytes of workers potentially exposed to zineb and/or mancozeb have been carried out **(outlined in Section 3.3.5.2.3)**. However, due to limitations in the design and reporting of the studies no firm conclusions could be drawn from them. No studies were available in which the carcinogenic or reproductive toxicity potential of zineb in humans.*

Risk Assessment for human health

The mammalian toxicology of zineb was considered in 1999 and the ACP agreed that the NOAEL of 3 mg kg^{-1} d^{-1}, derived from the 90 d study with mancozeb in the dog, should be used in the risk assessments for systemic toxicity to professional operators (including chandlers). The ACP agreed that this could be used as an equivalent NOAEL for zineb, in accordance with the discussions in Section 4 of the main document. This NOAEL appeared also to be compatible with the available data on ETU toxicity, taking into account the approximate 20 % metabolism of zineb to ETU. The exposure of amateur users applying antifouling product was considered to be very short term and very infrequent and therefore comparison with endpoints for acute toxicity (using the oral LD_{50} of 7000 mg kg^{-1}) was considered appropriate.

Section 3.2.1.2.1 reports dermal penetration of less than 1 % for an aqueous suspension of zineb applied to rat skin. However, no information is available on the skin penetration of zineb (or mancozeb) when in solvent-based formulations such as antifouling products. For the purposes of risk assessment a value of 10 % penetration has been assumed. The skin sensitization potential of zineb has been considered for both professional and amateur operators.

Data generated by the manufacturer and by HSE estimate similar levels of exposure of professional workers during spraying. The risk assessment highlights a potential cause for concern. However, exposure calculations have been based on an estimated 10 % skin penetration of zineb as explained above. The ACP considered that the known greater tolerance of humans to thyroid stimulation (stimulation in the human thyroid, e.g. in iodine deficiency, does not normally lead to tumours) suggested that the NOAEL selected for repeated exposure could be viewed as conservative.

In 1999, when the ACP considered the risk assessment, they agreed that approval holders should be required to carry out a study of skin penetration in order to enable the exposure estimates to be refined. In the interim it was agreed that further personal protective equipment (PPE), including respiratory protective equipment (RPE) be specified to mitigate the estimated exposures. Fuller details are given in Appendix 1. The higher TER values calculated using a figure of 1 % penetration of clothing to take account of such PPE, confirm that the requirements are necessary. In addition to the PPE specified previously, it is proposed that professional users should be required also to wear impervious footwear that protects the lower leg. This is good practice and is consistent with the requirements for other active ingredients used in antifoulings.

While amateur products have been approved for spraying, there is no information to indicate that this happens. The TERs calculated indicate that short-term exposure to zineb would not give rise to concerns for systemic toxicity. However, the partial review in 1999 identified that there might be concerns due to the potential for skin sensitization and possibly for respiratory irritation. At that time, the ACP considered that zineb might be considered a weak skin sensitizer but that the data on human experience did not indicate any grounds for serious concern. No data provided to this review suggested concerns for respiratory irritation. Therefore spray application of zineb-containing antifouling products was permitted to continue. However, exposure during spraying is likely to be greater than during application by brush and roller and the ACP therefore considered that amateur application by spraying should be revoked.

Exposure of professional workers and amateurs during application by brush and roller has been estimated using data generated by HSE. Systemic exposure of amateurs is considered acceptable. The potential for intake of zineb by inhalation during application by roller or brush is considered to be low and the risk is considered acceptable. However, there will be dermal exposure, whether or not amateurs wear workwear. Application of zineb-containing antifouling products by brush and roller was permitted to continue in 1999 because human experience indicated that the risk of sensitization was likely to be low. The ACP agreed that this remained acceptable provided that users are required to wear gloves as a precautionary measure.

Systemic exposure of professionals (chandlers) is considered acceptable at the central tendency but gives cause for concern at the worst-case level. As agreed in 1999, The ACP

has previously agreed that a better estimate of skin penetration of zineb from antifouling products would enable the risk assessment to be refined. In the interim, it was agreed that chandlers applying zineb-containing antifoulings by brush and roller should be required to wear a standard of personal protective equipment equivalent to that used by other professionals. However, the potential for intake of zineb by inhalation during application by roller or brush is considered to be low and the RPE is not considered necessary.

No data are available for bystander exposure to antifoulings. It is considered that bystander exposure to antifouling products applied to the hull of large commercial vessels will be lower than for other workers, since spraying operations are generally avoided by others in the dry-dock, vessels do not emit zineb once antifouling product has settled on the ship surface and contact with wet surfaces by third parties is unlikely. For amateur applications, passers-by within a congested yard could contact the hulls of treated boats. There is some risk of skin sensitization for bystanders if such exposure were repeated. However, the ACP have stated previously that they consider the risk of sensitisation by zineb to be low.

Environmental Hazard

A number of companies supplied environmental hazard data in support of the use of zineb. A single company supplied data on the EBDC (ethylene bisdithiocarbamate) pesticide mancozeb together with a written argument that the structure and properties of zineb and mancozeb were similar enough to justify read-across between the two when assessing the environmental hazard of zineb. This reasoned case has been accepted the justification for read-across, and the company may use the mancozeb data to support their use of zineb as an antifoulant. However, at the ACP's Environmental Panel it was agreed that the risk assessment for zineb required robust data showing the fate and behaviour of the a.i. (active ingredient) and metabolites in marine water-sediment systems, and neither the currently available zineb or mancozeb data were considered sufficient to do this. Therefore, only the zineb data have been used when considering the risk of this a.i. to the environment.

Zineb:

Zineb hydrolysed readily, with a half-life of 96.1 h at pH 7, 18 - 20 ^{o}C. Several metabolites, including DIDT (5,6-dihydro-3H-imidazo (2,1-c) 1,2,4-dithiazole-3-thione) and ETU (ethylene thiourea), were produced in varying amounts, depending on the pH at which the reaction occurred. In seawater in the presence of copper, the main metabolite was EDI (ethylene diisothiocyanate), rather than ETU. Leaching rates ranged from 0.04 to >10 µg zineb cm^{-2} d^{-1} in distilled and seawater. This wide range possibly reflected the difficulty in measuring zineb in solution due to its instability. The known metabolites, DIDT and EDI, were detected in seawater leachate, supporting the conclusion that zineb degraded very quickly after leaching into the water. No information was provided on the behaviour of zineb in sediment.

Zineb was toxic to freshwater algae with a 96 h EC_{50} (inoculum) of 1.8 mg l^{-1}. The acute toxicity of zineb to freshwater and marine invertebrates appeared to vary widely, with L/EC_{50} values of 0.97 - >200 mg zineb l^{-1}. The acute toxicity to freshwater and marine fish was slightly less varied, with 96 h LC_{50} values ranging from 7.2 - 42 mg zineb l^{-1}. The most acutely sensitive aquatic species was Daphnia magna with a 48 h EC_{50} of 0.97 mg zineb l^{-1}. Zineb had high chronic toxicity to invertebrates, with a 21 d EC_{50} of 0.089 mg l^{-1} for D. magna; effects on intrinsic rate of natural increase and carapace length were observed.

Zineb was shown to be toxic to some micro-organisms in soil, with an EC_{50} (inhibition of nitrification) of 3.6 and 16 µg a.i. g^{-1} dry weight of soil depending on the soil type. This indicates a potential for zineb to also be toxic to micro-organisms in sewage treatment works and aquatic ecosystems. No data were provided on the toxicity of zineb to higher plants or sediment dwelling organisms.

Metabolites:

The metabolite DIDT appeared to be as toxic or more toxic to aquatic organisms than zineb, with an algal 96 h EC_{50} (growth) of 0.18 mg l^{-1}, an invertebrate 48 h EC_{50} of 0.21 mg l^{-1} and a fish 96 h LC_{50} of 0.49 mg l^{-1}. The algae Chlorella pyrenoidosa was the most sensitive species. DIDT also had high chronic toxicity to invertebrates, with a 21 d EC_{50} of 0.073 mg l^{-1} for D. magna; effects on intrinsic rate of natural increase and carapace length were observed. ETU, however, appeared to be generally less toxic to aquatic organisms than zineb, with an algal 96 h EC_{50} (yield) of 860 mg l^{-1}, an invertebrate 48 h EC_{50} of 26.4 mg l^{-1} and a fish 96 h LC_{50} of 7500 mg l^{-1}. D. magna was the most sensitive species. ETU had slightly lower chronic toxicity to invertebrates, with a 21 d EC_{50} of 18 mg l^{-1} for D. magna; again with effects on intrinsic rate of natural increase and carapace length observed. No data were obtained on the toxicity of the metabolite EDI. Also, no data were obtained on the toxicity of metabolites to higher plants or sediment dwelling organisms.

***Mancozeb as a model for zineb**:*

Mancozeb was also readily hydrolysed; half-lives of 2.2, 5.5 and 14.1 h, at pH 5, 7 and 9 respectively at 25 ^{o}C, were derived, and several metabolites, including DIDT and ETU, were detected. Hydrolysis was suggested to be the main route of degradation in aquatic systems, and was so rapid that mancozeb was not found in the sediment compartment. ETU also degraded readily with a half-life of 4 - 6.3 d in water. This metabolite was found in the sediment, but also degraded readily here with a half-life of 2 - 6.4 d. Mancozeb metabolites were shown to be relatively immobile to mobile. However, no information on the mobility of the parent compound in sediment was submitted. Information on the leaching of mancozeb from a treated surface was not submitted.

The acute toxicity of mancozeb to freshwater and marine invertebrates varied slightly, with L/EC_{50} values from 0.067 - 2.01 mg l^{-1}. The acute toxicity to freshwater fish was less varied, with LC_{50} values of 0.07 - 0.4 mg l^{-1}. The mysid shrimp was the most sensitive species, with a 96 h LC_{50} of 0.067 mg l^{-1}. Mancozeb had high chronic toxicity with a 28 d LOEC (survival) of 0.00456 mg l^{-1}. The toxicity of mancozeb to soil micro-organisms varied greatly with MICs (minimum inhibitory concentrations) ranging from $\leq$2 to >1000 µg l^{-1} depending on the species. Therefore, the potential for toxic effects to micro-organisms in sewage treatment works and aquatic ecosystems is unknown. No data were provided on the toxicity of mancozeb to algae, higher plants or sediment dwelling organisms.

Environmental Risk assessment

The risk assessment has concentrated on the marine environment since the data available are predominantly for the use of antifouling products (AFPs) in estuarine and coastal areas, although the risk to freshwater environments has not been precluded. However, the strategy for assessing risk to the marine environment is less well developed than for terrestrial or freshwater. Therefore, the risk assessment strategy for the purposes of the current review has

been presented in a supporting document 'Environmental Risk Assessment of Booster Biocides in Antifouling Products' (ACP 2002). This document presents a comprehensive and comparative risk assessment for all approved booster biocides and has been endorsed by the ACP's expert Ad-hoc Environmental Panel. Below are the main points of the risk assessment concerned with the use of AFPs containing zineb and detailed in 'Environmental Risk Assessment of Booster Biocides in Antifouling Products' (ACP 2002), however, reference to the complete document is advised.

Environmental concentrations

Usage information was taken from a survey conducted by the Environment Agency (EA) in 1998. The EA survey demonstrated that zineb was not significantly used in AFPs for pleasure craft in the UK (< 3 %). No measured data are available for zineb. However, the current monitoring data could only ever represent the usage levels for 1998, and predictions of maximum PECs were considered since post approval usage cannot be controlled. Therefore, PEC data based on 100 % usage (all pleasure vessels treated) of zineb AFPs were calculated using a model developed as part of a HSE/EA commissioned research project. The model, REMA (Regulatory Environmental Modelling of Antifoulants), is a steady-state QWASI (quantitative water air sediment interface) model, designed to predict concentrations of biocides in both the water and sediment compartments of estuaries/marinas/harbours. The mean PEC data exceeded the highest limit of detection quoted by CEFAS (> 5 ng l^{-1}) for all booster biocides. However, the mean PEC calculations for the sediment compartment were very low in all cases. Therefore, since no limit of detection has been made available specifically for zineb it is not known at present whether the levels could be detected (no analytical technique was developed for zineb).

Predicted no effect concentrations

Once released into the aquatic compartment, the chemical fate of the booster biocide will determine whether the toxic effect exerted is limited to the target organisms within a boundary layer of a painted surface or whether the a.i. persists and there is potential for exposure of non-target organisms. Therefore, selection of key non-target organisms and likely duration of exposure is essential, but this is somewhat reliant on the availability of acceptable data for representative marine species. Chronic data endpoints have been selected as more appropriate for the purpose of a marine risk assessment following the use of booster biocides. This is because the inputs of booster biocides into the marine environment as a result of leaching from multiple point sources (treated surfaces) will be a continuous process. Comparisons between marine and freshwater chronic toxicity data for booster biocides have not demonstrated any differences in sensitivities. Therefore, freshwater data have been accepted and the most sensitive species selected, regardless of test medium. The provision of safety factors has been made in accordance with the guidance detailed in the European Risk Assessment Technical Guidance Document (EURATGD, 1996), and those previously agreed by the ACP. The most sensitive species to chronic zineb exposure was shown to be <u>D. magna</u> . No company submitted chronic data were available, therefore, in light of the limited available data a 1000 safety factor was applied, and a PNEC of 0.018 μg a.i. l^{-1} given based on a 21 d LOEC for <u>D. magna</u>.

Risk quotient

The PEC_{water}:PNEC calculations derived for zineb at both low (3 %) and maximum (100 %) usage levels resulted in unacceptable PEC:PNEC ratios for both estuary and open marina sites. The REMA predicted zineb data indicate that refinement of the current risk assessment is necessary, because the present level of data submitted does not allow a conclusion to be reached regarding the risk to the aquatic environment (from either zineb or any subsequent metabolites). However, with additional data to support the rapid degradation of zineb and clarification as to the routes of metabolism in the aquatic environment, the risk assessment can be refined. The $PEC_{sediment}$ data derived by the REMA model suggest that the sediment compartment is not a concern for zineb. Therefore, no additional data to address zineb's behaviour or toxicity in sediment have been requested.

Efficacy

No laboratory screening data were submitted. The majority of the relevant Approval Holders provided efficacy test data generated using raft tests. The studies were conducted on a range of formulations. In the case of the TBT ablative formulations, it was possible to determine that these were representative of current approvals with respect to active substance levels of the organic 'booster biocide' zineb. The biocide was present in combination with copper (Cu_2O) as the principal biocide.

Studies were provided in support of antifouling products formulated using TBT and TBT-free ablative technologies. No data were provided specifically in support of products based on the soluble (conventional) matrix or contact leaching systems.

The raft studies were performed over periods of one or more 'seasons' at a number of test sites at widely differing geographical locations. The fouling challenges were equally diverse.

It can be concluded, from the various raft test studies, that the products based on TBT and TBT-free ablative coatings that contained zineb as a 'booster biocide' plus copper as either the sole principal biocide or in conjunction with other principal biocides achieved a satisfactory level of antifouling activity against both algae and animals. This performance was against a diverse fouling challenge that, depending on test site and environment, could often be severe. From the data provided, however, it was not possible to determine if the levels of zineb used in the TBT-free ablative formulations were representative of current approvals.

No field data (such as vessel patch or panel tests) were submitted by any of the Approval Holders in support of their products.

It should be noted that, particularly for ablative technologies, a static raft test provides a harsh challenge since effects due to physical ablation and/or polishing are minimised. For these types of coatings it can be expected that antifouling performance will be further improved on a moving vessel due to the ablative/polishing effects.

Overall recommendations

The ACP recommended that approvals should be allowed to continue for amateur and professional use containing maximum formulation concentrations of 20 % w/w zineb.

Certain restrictions have been placed upon this continued use (which include removal of amateur use by airless / conventional spray due to concerns over the skin sensitisation potential of zineb) plus additional data requirements. These restrictions and requirements are outlined fully in Section 9. These recommendations have been agreed by Ministers.

1 INTRODUCTION AND REGISTRATION HISTORY

1.1 INTRODUCTION AND BACKGROUND TO THE REVIEW

Zineb is one of a group of compounds known as the ethylene bis-dithiocarbamates (EBDCs). It is used as a fungicide in agriculture and is also used in approved antifouling products.

1.1.1 EARLIER REVIEW OF AGRICULTURAL USE

A partial review of the use of EBDCs in agriculture only was carried out in 1989-90 (ACP 1991). This concentrated on the risks to consumers arising from food residues of the parent EBDCs and of ethylene thiourea (ETU), a common contaminant and metabolite of EBDCs. Data on metabolism, carcinogenicity, mutagenicity and developmental effects were examined. The ACP agreed that the findings in studies with the EBDCs were all attributable to the effects of ETU. There was no evidence that ETU or the EBDCs were mutagenic *in vivo*. The major finding in rodents was an alteration in thyroid hormone levels, considered to be due to the inhibition by ETU of iodide oxidation. This, if persistent, led to the production of thyroid tumours. However, such tumours were considered to regress rapidly when dosing ceased. It was also noted that rats were more sensitive to thyroid stimulation and that stimulation in the human thyroid (e.g. in iodine deficiency) did not normally lead to tumours. The ACP agreed that only continuous exposure to ETU at a dose that persistently elevated serum levels of thyroid stimulating hormone (TSH) presented any risk. A 90 d study in the rat established a no-effect level of 1.78 mg ETU kg^{-1} d^{-1}, with elevation of serum TSH observed at higher doses. In addition, a no-effect level of 15 mg ETU kg^{-1} d^{-1} was identified, based on an increased incidence of liver tumours at higher dietary levels in a two-year mouse study. The ACP considered levels of the EBDCs in the diet and the potential for their metabolism to ETU and the occurrence of thyroid and liver tumours. It concluded that there was no evidence of a risk of cancer or other adverse health effects to consumers as a result of the agricultural use of EBDCs.

Sections of ACP 1991 evaluating data on ETU, mancozeb and zineb and commenting on the possible relevance of particular effects were considered during this evaluation.

1.1.2 REVIEW OF ANTIFOULING USE

Following reviews of the use of triorganotins and copper compounds in antifouling products, Ministers agreed in 1995 that reviews of all the 'booster biocides', the organic active ingredients used in association with copper and/or triorganotins, should receive priority. The ACP agreed a timetable to complete the hazard evaluations of these substances by 2000 so all of the reviews could be considered together and a concerted approach to the environmental risk assessment undertaken.

To ensure that a consistent approach was applied to all the booster biocides, it was recommended that overviews of the available mammalian toxicology be prepared for each booster biocide. Having examined these overviews in May 1997, The ACP required that the mammalian toxicology of those booster biocides that might be noted as being of concern for human health should be reviewed urgently. Zineb was one of these compounds.

The ACP considered an evaluation of the physicochemical and mammalian toxicology data submitted to the zineb review in October 1999. None of the data submitted by the manufacturers had previously been considered by the committees. A literature survey also had been carried out and relevant information evaluated. The data provided by the manufacturer included studies on the related compound mancozeb and the ACP accepted the manufacturer's argument that the toxicological data could be used to 'read-across' to zineb.

The ACP agreed the that provisional approval should be permitted to continue for zineb-containing antifouling products, subject to data requirements addressing analytical and physicochemical endpoints and the skin penetration of zineb in antifouling products. Personal protective equipment was specified as a condition of approval. Full details are set out in Appendix 1. These requirements were conveyed to approval holders but at the time that this review was reconsidered the deadline for data submission had not yet expired.

This paper includes the evaluation of physicochemical and mammalian toxicology data on zineb that was considered in 1999. The assessment of risk to users and bystanders has been revised, as requested by the ACP, to take into account all factors affecting exposure and increase the clarity and transparency. In addition, the document includes an evaluation of the available environmental and efficacy data on zineb. The environmental data on each of the booster biocides have previously been considered by the Environmental Panel of the ACP, together with an assessment of the risks to the environment. The environmental risk assessment for each of the booster biocides has been presented in one overall document and is made available separately (ACP 2002).

1.2 REGISTRATION HISTORY OF ANTIFOULING PRODUCTS

1.2.1 BACKGROUND

In July 1987, antifouling products were brought under The Control of Pesticides Regulations (COPR), 1986. As a result of discussions between Government Departments and the antifouling product industry, it was agreed that these products would not be subject to the same registration procedures as other non-agricultural pesticides until their active ingredients were fully reviewed. Companies producing antifouling products had been invited in April 1987 to identify all active ingredients used in the formulations that they wished to be included under the Regulations - although many of these had not been previously evaluated by the ACP, there was no requirement at that time to submit any safety or other data to support their applications.

In addition, antifouling companies were permitted to seek approval for products where, unlike other pesticide products, the active ingredient(s) was specified in terms of a percentage range instead of a fixed value and that there were no restrictions placed upon the number of active ingredients permitted in each antifouling product.

It had been further agreed, and published in The Edinburgh Gazette of Friday 26th June 1987 to this effect, that antifouling products should be classified and labelled in accordance with the 77/728/EEC Council Directive relating to classification, packaging and labelling of paints, varnishes and inks (European legislation which has subsequently been superseded by the 88/379/EC Council Directive relating to the classification, packaging and labelling of

dangerous preparations). As an outcome of this decision with respect to labelling of antifouling products, it was only necessary for the names of certain 'dangerous substances' to appear on product labels rather than each active ingredient and its percentage in w/w.

The first approvals of antifouling products containing zinc ethylene bis-(dithiocarbamate), known more commonly under its BSI name of zineb, were given under these transitional arrangements.

In 1991, proposals attempting to terminate the transitional arrangements granted to antifouling products was presented to the Committees, so that all pesticide products would be dealt in a similar fashion. However, it was considered prudent to leave these arrangements in place until a full review of all active ingredients found in antifouling products had taken place.

1.2.2 PROPOSED INTERNATIONAL BAN ON ORGANOTINS

On the 22nd of November 1999 the IMO General Assembly passed a resolution, put forward by the Marine Environment Protection Committee (MEPC), that the MEPC should develop a global, legally binding, instrument to address the harmful effects of anti-fouling systems used on ships. The assembly also agreed that the instrument should ensure a global prohibition of the application of organotin compounds by 1 January 2003 and the prohibition of its presence on ships by 2008. At present there is no guidance on the definition of 'organotin compounds' and whether this covers both copolymer and free-association systems.

A working group within MEPC has started to draft a freestanding international convention to regulate shipboard antifouling systems that have adverse effects on the marine environment. This will include an annex of systems subject to specific controls, including a ban on their use. IMO Council agreed that there should be a Diplomatic Conference on the convention in 2001.

It should be noted that any ban on the use of organotins in antifouling products could result in increased use of any or all of the booster biocides.

1.2.3 CURRENT PRODUCTS CONTAINING ZINEB

As of November 1999, there were 270 approved antifouling products that could be marketed legally in the UK. Of these, 32 (approximately 11.9 %) actually contained zineb as one of their active ingredients.

Zineb is permitted in approved professional and amateur antifouling products for use on vessels of any size plus structures below the waterline such as oil platforms, jetties, navigation buoys or piers. It cannot be used in aquaculture (on apparatus or equipment used in the cultivation of fish and shellfish plus fishing nets, lobster pots etc). The maximum concentrations of zineb permitted as an active ingredient within approved formulations are:

Products applied to vessels by amateurs: 20.0 % w/w
Products applied to vessels by professionals: 20.0 % w/w

At present, of the 32 currently approved antifouling products containing zineb,

5 products are for amateur and professional use;
27 products are solely for professional use.

The distribution of zineb antifouling products by active ingredient is shown in Table 1.1.

Table 1.1 The distribution of zineb antifouling products by active ingredient

Active ingredient(s)	No.	Active ingredient(s)	No.
Zineb/Cu_2O	4	Zineb/Cu_2O/TBTM/TBTO	17
Zineb/Cu_2O/copper resinate	1	Zineb/CuSCN/TBTM	2
Zineb/Cu_2O/TBTM	6	Zineb/CuSCN/TBTM/TBTO	2

The current maximum level in existing products is 18.0 % w/w (Unpublished, 1999a, b, c).

Application rates recommended by companies for products containing zineb vary depending upon the expected service life of the antifouling coating. For example, products applied at a rate of 1 l per 4 m^2 will require repainting every 24 - 36 months, whilst those applied at a rate of 1 l per 2 m^2 will require repainting every 60 months.

Acceptable application methods for applying approved products containing zineb to vessels/structures are by brush, roller and airless or conventional spray. Applying antifouling product by spray requires 2 to 4 persons per spray position. The three identified tasks are:

- spraying - the sprayer,
- mixing and loading - the pot-man (who prepares the antifouling product and ensures its supply to the high pressure pump),
- ancillary - the rein or tender men, who attend to keeping paint lines free and may also manoeuvre the mobile access platform (cherry-picker).

1.2.4 CLASSIFICATION AND LABELLING OF ANTIFOULING PRODUCTS CONTAINING UP TO 20 % W/W ZINEB

1.2.4.1 BACKGROUND

Under transitional agreements reached between Government Ministers and industry in 1987, antifouling products are classified and labelled as if they were paints and must comply with current EC legislation, namely Council Directive 88/379/EC relating to the classification, packaging and labelling of dangerous preparations (which is currently implemented in the UK as The Chemicals (Hazard Information and Packaging for Supply) Regulations 1994).

This 'Dangerous Preparations' Directive (and its subsequent adaptations) allows EC classifications assigned to each substance within a preparation to be pooled together in order to determine overall EC Classifications for the product, by use of an agreed set of calculations and assumptions as laid out in an annex to this Directive.

The EC classification(s) for each substance used in this determination of product classification must be taken, whenever possible, from a European inventory - Annex I to Council Directive 67/548/EC relating to the classification, packaging and labelling of

dangerous substances. This Annex contains EC classifications for a number of compounds/elements that have been agreed by EC Member States after consideration of all available toxicological and environmental data. Therefore, where substances have already been considered by EC Member States, decisions taken on their classification(s) will override any such decisions taken at a national level.

If a substance does not have an entry on Annex I of Directive 67/548/EEC, then data provided on the manufacturer's Material Safety Data Sheet for that substance should be used in determining overall classification.

However, it should be pointed out that should actual formulation data be available, then this will override any determination of product classification which has been derived from the classifications of its component substances.

1.2.4.2 CLASSIFICATION/LABELLING UNDER UK LEGISLATION

Zineb itself has an entry on Annex I to Directive 67/548/EEC (reproduced in the UK as various editions of The HSC Approved Supply List) stating that it is classified as:

SENSITISING (Xi): R43: MAY CAUSE SENSITISATION BY SKIN CONTACT.
IRRITANT (Xi): R37: IRRITATING TO RESPIRATORY SYSTEM.

At present, Council Directive 88/379/EEC is implemented as The Chemicals (Hazard Information and Packaging for Supply) Regulations 1994 - CHIP 2. Recent adaptations to the Directive have been introduced into the UK as amendments to CHIP 2, the latest set being presented as CHIP 99.

Currently no environmental labelling for products is required under CHIP. However, it is to be introduced in the future and antifouling products will be labelled in line with the consequent requirements.

Therefore antifouling products containing 1.0 - 19.9 % w/w of zineb would attract the following classification under CHIP 99:

EC Classification: SENSITISING;
Hazard symbol: IRRITANT (ST ANDREW'S CROSS);
Risk phrase: R43: MAY CAUSE SENSITISATION BY SKIN CONTACT.

Antifouling products containing 20.0 % w/w of zineb would attract the following classification under CHIP 99:

EC Classification: SENSITISING and IRRITANT;
Hazard symbol: IRRITANT (ST ANDREW'S CROSS);
Risk phrase: R43: MAY CAUSE SENSITISATION BY SKIN CONTACT.
R37: IRRITATING TO RESPIRATORY SYSTEM.

1.2.5 TYPICAL FRAME FORMULATION OF A YACHT ANTIFOULING PAINT

Typical yacht paint consists of biocide and pigment dispersed in a resinous binder, reduced to an acceptable application viscosity with solvent. Additives are incorporated to modify paint film properties, application or storage characteristics.

2 PHYSICAL CHEMISTRY

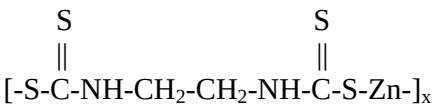
2.1 INTRODUCTION

All the data in this Section were presented in late 1999 to the ACP. Approval holders have been allowed 1 year to address the data requirements identified, as set out in Section 2.6. No additional information had been provided at the time this review was reconsidered in 2000.

Identity, physico-chemical properties and analytical methods for zineb have been summarised. Some test reports for the physico-chemical properties have been submitted.

2.2 IDENTITY OF THE ACTIVE SUBSTANCE

Common name: Zineb
IUPAC name: Zinc ethylenebis(dithiocarbamate)(polymeric)
Synonyms or codes: (ISO - E) Zineb, ENT 14874
CAS number: 12122-67-7
EINECS number: 235-180-1
Molecular formula: $(C_4H_6N_2S_4Zn)_x$
Structural formula:



Molecular mass: $(275.8)_x$

Percentage purity:

1. Minimum 92 % w/w (Range 92 - 95 % w/w)
2. Minimum 94 % w/w (Range 94 - 97 % w/w)

Spectral data: The spectral data provided (UV and IR) are broadly consistent with the chemical structure of the compound [Unpublished, undated (b); Unpublished, 1987 (a)]. An acceptable reasoned case has been provided as to why zineb cannot be characterised using MS and NMR [Unpublished 1999(a)]

2.3 PHYSICO-CHEMICAL PROPERTIES

Physical state at 25 ^{o}C and 101.3 kPa:	Off-white solid [Unpublished, 1995 (b); Unpublished, undated (c)]
Melting point:	Decomposed before melting (at 157 ^{o}C)***** [Unpublished, 1995 (b)] [Tomlin, 1987]
Boiling point:	N/A

Relative density D_4^{20}:	1.96 [Unpublished, 1991]
Surface tension at 25 ^oC:	Not provided
Vapour pressure at 25 ^oC:	Calculated as $3.98x10^{-4}$ Pa [Unpublished, 1995 (b)] (Modified Watson Correlation) Negligible [Worthing, C.R and Walker, S.B, 1987, Bol *et al*., 1991] 9.7×10^{-8} [Ordelman *et al*., 1993] $<4.0 \times 10^{-4}$ [Unpublished, 1999 (b)]
Solubility in water at 20 ^oC:	0.07 mg l^{-1} [Unpublished, 1992] 10 mg l^{-1} [Bol *et al*., 1991, Luttik and Linders, 1990 (a), Tomlin, 1997] 1.2 mg l^{-1} (at 25°C) [Unpublished, 1987 (b)]
Partition Coefficient: log P_{ow} at 20 ^oC:	0.77 [Unpublished, 1987 (b)]
Flammability:	Highly flammable [Unpublished, 1995 (c)]

2.4 STORAGE STABILITY

No data have been provided.

2.5 ANALYTICAL METHODS

The following methods for the determination of zineb have been submitted:

1. Analytical methods for the determination of zineb in the technical material:

(i) Unpublished, 1994 (a).
(ii) Bighi, C., 1964.

2. Analytical method for the determination of inert ingredients in the technical material:

(i) Unpublished, 1997 (b).
(ii) Unpublished, 1994.
(iii) Unpublished, 1995 (d).
(iv) Unpublished, 1995 (e).
(v) Unpublished 1996 (c).
(vi) Unpublished, 1997 (c).

3. Analytical method for the determination of the active ingredient in aqueous solutions:

Unpublished, 1996 (b).

4. Analytical methods for the determination of zineb in formulations:

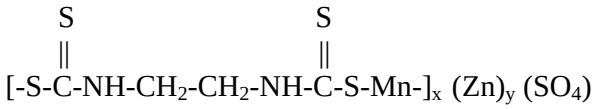
(i) Unpublished, 1993 (a).
(ii) (a) Unpublished, 1996 (c)].
(b) Unpublished, 1996 (c); Christensen, L. H, and Drabaek, I., 1983; Baccanti, M. and Columbo, B., 1989; Kirsten, W.J. and Hesselius, G.U., 1983.
(iii) Unpublished, 1993 (b).

2.6 DATA REQUIREMENTS:

Members agreed in 1999 that further physicochemical and analytical data (see Appendix 1) should be required. When the ACP considered this evaluation no data had yet been received. No additional data requirements have been identified.

2.7 PROPOSED READ ACROSS OF TOXICITY AND ENVIRONMENTAL DATA FROM MANCOZEB

One of the manufacturers has proposed that mammalian toxicity and environmental data on the related EBDC, mancozeb, can be 'read-across' to zineb. Mancozeb is stated to contain 20 % w/w manganese and 2.55 % w/w zinc. The structural formula of mancozeb is as follows:



Reasoned cases were provided. As part of the supporting argument, both test data and results from published studies for the water solubility, vapour pressure and octanol/water partition coefficient (log P_{OW}) have been submitted for mancozeb. Table 2.1 compares these data with the corresponding information for zineb. The physicochemical endpoints provided support the argument for 'read-across' from mancozeb to zineb.

Table 2.1 Physicochemical properties of zineb and mancozeb according to several sources

Parameter	Zineb	Mancozeb
Water solubility (mg l^{-1})	10 [Bol *et al*., 1991)	Practically insoluble [Bol *et al*., 1991]
	10 [Luttik and Linders, 1990a)	<0.1 [[Luttik and Linders, 1990b)
	10 [Tomlin, 1997]	6.2 [Tomlin, 1997]
	1.2 (at 25°C) [Unpublished, 1987 (a)]	2 [Unpublished, 1987 (c)]
	0.07 (at 20°C) [Unpublished, 1992]	0.67 [Unpublished, 1994 (b)]
Vapour pressure (Pa)	9.7×10^{-8} [Ordelman *et al*., 1993]	9.7×10^{-8} [Ordelman *et al*., 1993]
	'Negligible' [Bol *et al*., 1991]	'Negligible' [Bol *et al*., 1991]
	$<4.0 \times 10^{-4}$* [Unpublished, 1999 (b)]	$<5.6 \times 10^{-5}$* [Unpublished, 1994 (c)]
log P_{ow} (at 20 °C)	0.77 [Unpublished, 1987 (b)]	1.38 [Unpublished, 1987 (c)]

*Determined using the estimation method.

3 MAMMALIAN TOXICOKINETICS AND TOXICOLOGY

3.1 INTRODUCTION

Data were provided on the active ingredient and a number of formulations containing 70 or 80 % zineb or a nominal 80 % mancozeb. The Sections of the 1990 partial review (ACP 1991) of the use of EBDCs in agriculture, which evaluated data on zineb, mancozeb, and their metabolite ETU and commented on the possible relevance of particular effects was considered as part of this evaluation. A reasoned case to support the read-across of data on mancozeb to zineb was also considered within this evaluation.

3.2 MAMMALIAN TOXICOKINETICS

3.2.1 STUDIES IN ANIMALS

3.2.1.1 ORAL

3.2.1.1.1 Zineb

3.2.1.1.1.1 Rat

An extensive series of investigations conducted to GLP on the toxicokinetics of zineb in Sprague-Dawley rats following oral administration is available in an unpublished report. For these studies, zineb was synthesised from $^{13/14}$C-ethylenediamine (EDA) and its dihydrochloride salt so that the label was situated on the ethylene moiety of the molecule. Throughout these studies zineb (both radioactive and non-radioactive) was administered by gavage as a suspension in a 1 % (w/v) aqueous sodium carboxymethylcellulose solution unless stated otherwise. It is not stated in the test report whether or not the animals were fasted prior to dosing, although it is likely that they were, as this is standard practice for studies of this nature. All values presented are group means unless otherwise stated (Unpublished, 1986a).

Single dose balance studies were conducted with ^{14}C-zineb administered at doses of either 5 or 50 mg kg^{-1}. Five male and 5 female rats were treated at each dose. Urine and faeces were collected (generally over 24 h intervals) for five days immediately following dosing. Animals were then killed and a wide range of organs and tissues assessed for radioactivity; distribution and metabolism studies were also carried out using these tissues and excreta. Recovery of radioactivity was complete (99 - 103 % of the administered dose) at both dose levels and in both sexes at the end of the study period. Most of the recovered radioactivity (~95 %) was found in the urine and faeces, at both doses, with no difference between sexes. Slightly more radioactivity (between 51 and 56 %) was excreted via the urine than eliminated in the faeces (40-47 %); this pattern was the same at both dose levels in both sexes. Much of both the urinary (88-92 % of the radioactivity excreted via this route) and faecal (72-80 % of

the radioactivity eliminated via this route) elimination of radioactivity occurred over the first 24 h after dosing, with excretion via these routes essentially complete, apart from trace amounts, after 48 h. This pattern of excretion was seen at both dose levels with a slightly slower rate seen at the higher dose. The remaining radioactivity was found in the carcass.

As a preliminary to these balance studies a smaller scale study was carried out on 2 males and 2 females given 50 mg kg^{-1} ^{14}C-zineb. Urine and faeces were collected over the same time period as that in the main study. In addition, expired air was collected daily for three days after dosing and assessed for radioactivity. As in the main study, excretion via the urine and elimination via the faeces accounted for most (~95 %) of the administered dose that was recovered (88 % recovered for males, 82 % for the females). Only a small amount (~2 %) was excreted via expired air; the form in which the ^{14}C was present in the expired air was not determined.

A repeat dose balance study was also conducted on 5 male and 5 female rats which received 5 mg kg^{-1} d^{-1} non-radiolabelled zineb for 14 d. On the 15th day each rat received 5 mg kg^{-1} ^{14}C-zineb after which urine, faeces, organs and tissues were collected as for the single dose balance study. Essentially similar results were obtained as for the single dose study, with most radiolabel being excreted via the urine (around 50 % of the administered dose) and faeces (40-45 % of the administered dose) mainly within 48 h after the radioactive dose; no sex difference in excretion was seen.

The uptake of radiolabel into the plasma following oral dosing was also investigated in a separate part of the same study. Five rats of each sex were administered a single oral dose of either 5 or 50 mg kg^{-1} ^{14}C-zineb. Tail vein blood samples were taken pre-dosing and then from between 15 minutes and 168 h after dosing. Plasma was separated and the radioactivity determined with the results expressed as µg equivalents ^{14}C-zineb m^{-1} plasma. No difference was seen between sexes in the pattern or time course of the results obtained. At both dose levels, plasma radioactivity increased rapidly reaching peak levels at 4 h after dosing with a subsequent rapid decline. In the plots of the plasma radioactivity concentration against time, the mean values for the area under the curve were slightly greater in females (by ~20 %) than males at both doses and were approximately 10-fold greater after the higher dose compared to the lower in both sexes, indicating that the extent of uptake as a proportion of the dose was the same for both doses.

The potential for biliary excretion was investigated as another part of the same series of investigations. For this groups of 3 male and 3 female rats with indwelling bile duct cannuli received either 5 or 50 mg kg^{-1} ^{14}C-zineb and a balance was performed. Bile was collected over 90 min periods for up to 48 h after dosing. Urine and faeces were collected over each of the 2 days. After 48 h the animals were killed and the radioactivity measured in the GI tract, liver and carcass. Most (between 94 and 99 %) of the administered radioactivity was recovered at both doses in both sexes. As seen in the other studies, much (~80-90 % of the administered dose) of the radioactivity was excreted in the urine and faeces over the 48 h of the study period. In this particular study slightly more radioactivity (~62 % of the administered dose in both sexes) was excreted via the urine at the higher dose. Biliary excretion, mainly within the first 24 h after dosing, accounted for around 5-6 % of the administered radioactivity in both sexes at both dose levels. Around 1-2 % was found in the GI tract with between 0.2 and 1 % in the liver, at 48 h. The biliary excretion results indicate that much of the faecal radioactivity recovered in rats following oral dosing resulted from

administered substance passing directly through the gastrointestinal tract, with only a small percentage representing excretion of absorbed material.

Qualitative (autoradiography) and quantitative (scintillation counting) tissue distribution studies were also undertaken. Quantitative tissue distribution data were obtained from the balance studies described above. Further to these, groups of 5 male and 5 female rats received 5 mg kg^{-1} ^{14}C-zineb daily for 7 d and were then killed at 4, 24, 72, 120 and 168 h after the last dose. Radioactivity was measured in a full range of tissues, including blood taken by cardiac puncture. For the qualitative study, 5 male rats received a daily dose of 5 mg kg^{-1} ^{14}C-zineb daily for 7 d; a further male received only a single dose. The rat receiving the single dose was killed 24 h after dosing and the remainder at 4, 24, 72, 120 and 168 h after the last repeated dose. Autoradiographs were prepared of 6 sagittal sections obtained between the level of the kidney and spinal cord from each animal.

Quantitatively, tissue distribution was expressed either as the percentage of the administered radioactive dose present in total tissue or as tissue concentration in µg zineb equivalents g^{-1} tissue. As indicated above, for the balance studies at 5 d after a single dose of both 5 and 50 mg kg^{-1} only a very small amount (1-2 %) of the administered radioactive dose was retained in the body, though some was found to be present in all tissues sampled. When expressed as the percentage of the administered dose retained in total tissue the kidneys (~0.2-0.4 %), liver (~0.1-0.2 %) and muscle (~0.2-0.7 %) contained the majority of retained radioactivity. However, when expressed as the tissue concentration, the thyroid contained most (~10-13 µg zineb equivalents g^{-1} tissue after 5 mg kg^{-1} and 65-75 µg zineb equivalents g^{-1} tissue after 50 mg kg^{-1}) radioactivity, with the kidney, liver and adrenals having the next highest concentrations, approximately 5-10 fold less than in the thyroid. There was little difference in tissue distribution between the sexes. Essentially similar tissue distribution results were obtained in the balance study in which animals received non-radioactive zineb (5 mg kg^{-1}) for 14 d, followed by a single 5 mg kg^{-1} dose of ^{14}C-zineb and killed 5 d later.

Results from the animals that received a daily dose of 14C-zineb for 7 d indicated a similar tissue distribution in terms of tissue concentrations (not expressed as a percentage of the dose) as that seen after the single doses. As for single doses, at 4 h after the last dose, the thyroid (95 µg zineb equivalents g^{-1} tissue in males; 300 µg zineb equivalents g^{-1} tissue in females), kidney (17 µg zineb equivalents g^{-1} tissue in males; 27 µg zineb equivalents g^{-1} tissue in females), liver (3 and 4 µg zineb equivalents g^{-1} tissue in males and females) and adrenals (3.3 and 4.8 µg zineb equivalents g^{-1} tissue in males and females) contained the highest concentrations, though radioactivity was found in all tissues sampled. The GI tract also retained more (~50 µg zineb equivalents g^{-1} tissue in both sexes) than most internal tissues. At 24 h after the last dose the levels in tissues declined and continued to do so until the end of the study, although some activity remained in all tissues at that time. Although only one rat of each sex was sampled at each time point, the data indicated that the decline in radioactivity was generally slower in the thyroid and kidneys compared to other tissues. Furthermore the data also indicated that females retained slightly more radioactivity than males. Comparison of the data at an equivalent time (5 d) after the final radioactive dose indicates that the tissue concentrations following repeated dosing were generally greater (between 3 and 10 fold) than following a single dose at the same dose level. Allowing for the fact that the repeated dose data are based on fewer animals and hence less robust, nevertheless this suggests that there is some accumulation in tissues on repeated exposure.

The results of the quantitative tissue distribution studies were confirmed by the qualitative autoradiographic study. This indicated that the radioactivity taken up on repeated dosing was generally extensively distributed around the body. The autoradiographs revealed a relative tissue distribution similar to that indicated by the quantitative methods with the most intensive levels of radioactivity associated with the thyroid and kidneys. However, the autoradiographs also revealed radioactivity in the keratinised layer of the squamous epithelium of the tongue, oesophagus and non-fundic area of the stomach and also in the walls of the major arteries. Much of the radioactivity was associated with the excretory tissues, particularly the kidney. Enlargement of the autoradiograph of the kidney of the rat killed 24 h after the last of seven radioactive doses showed that the highest levels of radioactivity in this tissue were associated with the renal outer cortex with relatively much lower levels in the renal medulla. This pattern was representative of that seen throughout the post-exposure period. Comparison of the intensity of the exposures on the autoradiographs from the single and repeat dose animals at 24 h after the last dose indicated accumulation of radioactivity following repeated exposure, consistent with the quantitative information.

The metabolism of ^{14}C-zineb was also investigated using material collected from the above studies. Radioactive components in the urine collected over the first 24 h from rats that received a single dose of 5 or 50 mg kg-1 14C-zineb were investigated by thin layer chromatography (TLC) and mass spectrometry. Up to eight radioactive components were identified in the urine by TLC. Around 17-20 % of the radioactivity eliminated in the urine over this time (equivalent to around 8-9 % of the administered dose) at both dose levels in both sexes was identified as ethylenediamine (EDA). At 5 mg kg^{-1} around 40 % of the radioactivity in the urine (20 % of the dose) was identified as ethylenethiourea (ETU) in both sexes; this proportion was 25 % in males and 35 % in females of the radioactivity at 50 mg kg^{-1}. Ethyleneurea (EU) represented 9 % (in both sexes) of the urinary activity (~4.5 % of the dose) at 5 mg kg^{-1} but was slightly higher, 13-14 % (~7 % of the dose) at 50 mg kg^{-1}. Thiourea (TU) represented around 6-7 % of the urinary activity (~3 % of the dose) at 5 mg kg^{-1} in both sexes, and 4 % (~2 % of dose) in males at 50 mg kg^{-1} but was not detected in females at the higher dose. A further metabolite, N- acetylethylenediamine (N-AcEDA) accounted for 9-10 % of the urinary radioactivity (~4-5 % of dose) at 5 mg kg^{-1}; at 50 mg kg^{-1} it was difficult to detect the contribution of this particular metabolite as it co-eluted with another unidentified component. The remainder (~16 % at 5 mg kg^{-1}; ~40 % at 50 mg kg^{-1}) of the urinary activity was accounted for by further unidentified components. The presence of these metabolites, as well as a further one di-acetylethylenediamine, was confirmed qualitatively by nuclear magnetic resonance spectroscopy of a crude extract from the 24 h urine of rats which received ^{12}C/^{13}C-zineb at a dose of 50 mg kg^{-1}. It was demonstrated by TLC that no radioactivity in the urine was associated with oxalic or hippuric acid, glycine, urea or ethanolamine. Further TLC analysis was performed on 0-24 h urine, treated with glucuronidase and sulphatase, from male rats that received a single dose of 5 mg kg^{-1} ^{14}C-zineb. These treatments had no significant effect on the relative distribution of radioactivity between the different metabolite fractions, indicating no significant amounts of glucuronidation or sulphation had occurred.

Similar TLC analyses in order to identify potential metabolites were carried out on bile collected during the biliary secretion balance study and on the liver and kidneys from repeat dose investigations. Qualitatively similar metabolites were identified in the bile to those seen in the urine. Quantitatively, however, there were significant differences. More (between 31-37 % bile radioactivity; 1-2 % of the dose administered) of the radioactivity was as EDA, and less (between 13-16 % bile radioactivity; 0.5-1 % of the dose administered) with ETU at

both doses in both sexes. EU accounted for between 6-11 % of the bile radioactivity (0.3-0.7 % of dose) and TU and N-AcEDA each 6-9 % (0.3-0.6 % of dose) at both dose in both sexes. The remaining radioactivity was accounted for by unidentified components that were generally of a more polar nature than those found in the urine. Treatment with glucuronidase and sulphatase again had little effect on the distribution on the radioactivity between these metabolite fractions.

Liver and kidney samples were taken from the rats of both sexes (used for the quantitative tissue distribution study) which received 5 mg kg^{-1} d^{-1} ^{14}C-zineb for 7 d and which were killed at 4 and 24 h after the final dose. Again the qualitative profile of metabolites in both of these tissues was the same as in the urine and bile. In the liver at 4 h post-dosing, EDA accounted for ~40 % of hepatic radioactivity (~0.9 % of the administered dose) in both sexes; ETU for 26 % (0.5 % of dose) in males and 34 % (0.8 % of dose) in females; EU for 3-6 % (~0.1 % of dose); AcEDA for 3-8 % (0.05-0.2 % of dose); TU for 1 % (0.02 % of dose) with the remainder present in unidentified components. Roughly similar values were seen at 24 h after dosing for EU, TU and N-AcEDA but significantly more (66 % in males and 50 % in females) radioactivity was associated with EDA and less with ETU (9 % in both sexes). Glucuronidase treatment of the liver extracts from male rats sampled at 4 h post-dose resulted in a reduction of the radioactivity associated with EU (from 6 % to 1 %) and an increase in ETU (from 26 % to 40 %). The distribution of renal radioactivity within the metabolites was quantitatively similar to that seen in the urine at both 4 and 24 h post-dose in both sexes, with EDA accounting for 18-20 %, ETU 34-37 % and N-AcEDA for 8-10 %. The only difference was for TU and EU that accounted for around 1-4% at both times in male and females. Glucuronidase treatment of male rat kidney extract had little effect apart from a slight increase in EDA associated radioactivity (from 18 % to 23 %) and a slight decrease in ETU associated activity (from 37 % to 26 %).

In summary, the results from this extensive series of investigations demonstrate that when administered by oral gavage to rats around 50-60 % of the organic portion of zineb is taken up rapidly into the body. Unabsorbed zineb is rapidly eliminated in the faeces. Once absorbed, this is largely metabolised to a number of metabolites principally EDA and ETU. These metabolites and/or the organic portion of the parent molecule are widely distributed around the body with the thyroid a particular site for uptake. Most of the absorbed portion is eliminated from the body relatively rapidly within the first 24-48 h via the urine with small amounts (1 - 2 %) excreted in exhaled air and via the bile into the faeces. A small percentage is also retained in the tissues for several days post-dose. Although retention in tissues and organs of the organic component is a comparatively small amount in absolute terms there is some limited evidence that the parent molecule and/or metabolites can accumulate in tissues (particularly the thyroid) on repeated (and thus probably continuous) exposure.

In a published balance study, a group of two male and two female Charles River CD rats received a single oral dose of 50 mg kg^{-1} ^{14}C-zineb suspended in olive oil (Searle *et al*, 1987). The radiolabelled zineb was obtained commercially and labelled in the ethylene position. Following dosing the animals were housed singly in glass metabolism cages. Urine, faeces and expired air (for determination of $^{14}CO_2$) were collected over 24 h intervals up to 96 h after dosing, at which time the animals were killed and the carcass processed. The level of radioactivity in the excreta and carcass was measured and the presence and characterisation of metabolites in the urine and faeces determined by TLC. Data were presented as means of all four animals. Recovery of the administered radioactive dose was complete (107 %) at the end of the 96 h. Most of the recovered radioactivity (95 %) was found in the urine (63 %)

and faeces (32 %). Of the radioactivity eliminated via these routes most was excreted during the first 48 h after dosing (98 % of the total recovery in the urine and 89 % of the total recovery in the faeces). A small amount (3 %) of the administered radioactivity was recovered in the expired air as $^{14}CO_2$, again the majority (67 %) of this was within the first 48 h after dosing. The radioactivity remaining in the carcass accounted for around 1.5 % of the administered dose. Analysis of urine and faeces for metabolites revealed the presence of ETU, EU and unidentified polar material. ETU in the urine was found to account for ~19 % of the administered radioactivity, EU for ~5 % and polar material for ~13%. In faeces, ETU accounted for ~2 % of the administered radioactivity, EU for 0.3 % and the polar material for ~4 %. However, not all of the radioactive material was extractable from the excreta, with only about two-thirds recoverable from urine, one-third from faeces and thus around 50 % overall. Thus the amounts of metabolites present may have been underestimated. The pattern of excretion of the metabolites that were extracted was similar to the overall pattern of excretion of total radioactivity in that most was excreted within the first 48 h via both routes.

Aspects of the toxicokinetics of zineb were studied in rats following oral dosing in a published study (Camoni *et al*, 1984). A group of six (2 males, 4 females) rats obtained from Charles-River received a single oral gavage dose of 50 mg kg^{-1} zineb in olive oil. The zineb was a technical product of 89.5 % purity and containing <0.007 % ETU. A group of control animals (2 males, 4 females) received an equivalent volume of olive oil. Following dosing the animals were housed in metabolism cages. Urine was collected at 0-6, 6-24 and every subsequent 24 h period for 15 d after dosing. Faeces were collected daily for 3 d after dosing. ETU content was measured using a column extraction and gas chromatography with flame photometry technique developed specifically for the study. No ETU was found to be present in the urine of control animals. ETU appeared in the urine of the treated animals within the first 6 h after dosing with peak levels being reached at 24 h. In all around 65 % of the total urinary ETU collected over 15 d was excreted in the first 48 h. ETU continued to be excreted in low but measurable amounts over the remaining period of the study in three rats but could not be detected in the urine of the other three after day 7. Overall, the total urinary output of ETU was calculated by the Authors of the study to account for ~5 % of the administered dose of zineb. ETU was also detected in the faeces during the first 48 h after dosing but not in the following 24 h. Faecal elimination accounted for around 14 % of the total ETU excreted by both the urinary and faecal routes.

Another brief report provides an essentially qualitative description of the metabolism of ETU in rats following oral dosing (Truhaut *et al*, 1973). This report is of some significance since it is often cited as evidence that CS_2 is produced as a metabolite of zineb (and of EBDCs in general). It is in the form of a technical note and so only limited details are provided of the studies performed. Commercially available technical grade zineb (no further details provided) was used and purified in a series of water and organic solvent washes. The resulting material was suspended in distilled water and administered by oral gavage at a dose of 1000 mg kg^{-1} to a group of 50 Wistar rats (equal numbers of males and females), three times a week for two consecutive weeks. After the first dose two groups of three rats were used to collect expired air passed over activated charcoal over half-hourly intervals (the total collection time was unclear). The presence of CS_2 in the air was determined qualitatively by infrared spectrometry and was detected 2 h after dosing. No quantification of the amount (either absolute or relative to the dose administered) of CS_2 was reported, if attempted. The remaining rats were placed in metabolism cages, for the collection of urine and faeces, for the duration of the experiment, although only urinary metabolites are discussed further. Collected urine was extracted with chloroform and subject to chromatography. ETU and

ethylenebisthiocyanate were identified qualitatively as being present in urine after treatment as well as other unidentified metabolites. Again, no quantification of the amounts of these metabolites was presented in the report.

A description is provided, in a relatively old report, of an investigation of the toxicokinetics of zineb in rats following dietary administration (Blackwell Smith *et al*, 1953). The zineb used was described as a '65 % commercial wettable powder', although no further information was provided on its composition or purity. Furthermore, the analytical techniques used to determine zineb were not given though it was stated that the dietary levels were checked during the period of the experiment. Groups of 6 albino rats (strain and sex not stated) received diets containing either 396 or 9130 ppm of zineb for 3 d. Using accepted default values (Unpublished, 1990d), these dietary concentrations are estimated as equivalent to 39.6 and 913 mg kg^{-1} d^{-1}, respectively. Twenty-four hour food intakes were determined for each of the three days at each dose level. For the higher dose, starting from the beginning of the second day of dosing, faeces were collected over each of three successive 24 h periods. For the lower dose faeces were also collected but pooled as one 72 h sample. It is not stated in the report whether or not steps were taken to reduce contamination of the faeces with urine. Faecal samples only were analysed for zineb. It was stated that for the dietary dose of 396 ppm, about 69 % of the zineb intake was eliminated in the faeces; a slightly higher figure of about 75 % was reported for the 9130 ppm diet. Although not specifically stated how these values were calculated, they were presumably derived with knowledge of the daily food intake. Since zineb is known to be unstable in the presence of moisture the Authors speculated that degradation of the substance might have occurred during transit in the GI tract. To explore this possibility, the breakdown of zineb in the gastrointestinal contents from four untreated rats was investigated. A known amount of zineb ('approximating the faecal concentration') was added to the gastrointestinal contents and incubated at 39 ^{o}C for 24 h (estimated as the GI tract transit time). It was stated that an average 'loss' of 14.5 % of this material was observed. Bringing all these observation together, the Authors estimated that following dietary dosing only 11-17 % of administered zineb was absorbed. The results of this study are at odds with the relatively recent extensive oral toxicokinetics study. This may be due to differences in the dosing procedures (dietary administration versus gavage of suspensions) that may affect the uptake and bioavailability. However, there are numerous reporting deficiencies in the older study and thus overall less weight should be placed on those data compared to the more recent examination.

3.2.1.1.1.2 Mouse

A limited investigation of the toxicokinetics of zineb has been carried out in mice (Jordan and Neal, 1979). For this study ^{14}C-zineb was synthesised from ^{14}C-maneb and extracted twice with methanol immediately prior to dosing (presumably to ensure purity of the product). Though the purity of the final product was not given, it was stated that no ethylenebisdiisothiocyanato sulphide (EBIS), ETU or EU was present in the methanol extract as determined by chromatography. A group of three adult male ND/4(S) BR mice received a single dose of 0.25 mmole.kg^{-1} (~69 mg kg^{-1}) ^{14}C-zineb suspended in olive oil by oral gavage. The mice were then housed in a common metabolic chamber (hence all results were pooled values). Urine and faeces were collected over the following 0-24 and 24-48 h post-dosing. Expired CO_2 was trapped in NaOH over the same time periods. Radioactivity was determined in all three media. The 0-24 h and 24-48 h urine samples were pooled and analysed for metabolites using TLC.

Approximately 65 % of the radioactivity administered to the three mice was recovered at the end of the 48 h collection period from the urine and faeces. Though no data are presented it is stated by the Authors that no $^{14}CO_2$ was detected. Of the recovered activity, 96.5 % was excreted over the first 24 h collection period, mainly (90.4 %) in the faeces. TLC of the pooled urine samples demonstrated the presence of ETU (15.2 % of the radioactivity present in the urine) and EU (1 %). The majority (81 %) of the urinary radioactivity was accounted for by unidentified components more polar then EU. The remaining activity was present in unidentified components less polar than EU.

Although much more limited than the more recently conducted study in rats, these data from mice suggest some notable differences between the species. Although not all the radioactivity was recovered, there was apparently less uptake of the organic portion of zineb in mice than in rats. Elimination was mainly via the faeces in mice whereas there was a more even distribution between urine and faeces in rats. Excretion via expired air accounts for very little of the radioactivity excreted in both species, though only CO_2 output was assessed for mice and the position of the ^{14}C would not have allowed for detection of CS_2 described qualitatively in rats. Although less well studied in mice, metabolism studies identified ETU and EU as metabolites in both species.

3.2.1.1.1.3 Marmoset

In a published balance study, a group of two male and two female marmosets received a single oral dose of 50 mg kg^{-1} ^{14}C-zineb suspended in olive oil (Searle *et al*, 1987). The radiolabelled zineb was obtained commercially and labelled in the ethylene position. Following dosing the animals were housed singly in glass metabolism cages. Urine, faeces and expired air (for determination of $^{14}CO_2$) were collected over 24 h intervals up to 96 h after dosing, at which time the animals were killed and the carcass processed. The level of radioactivity in the excreta and carcass was measured and the presence and characterisation of metabolites in the urine and faeces determined by TLC. Data were presented as means of all four animals. Recovery of the administered radioactive dose was complete (99.4 %) at the end of the 96 h. Most of the recovered radioactivity (91 %) was excreted in the urine (53 %) and faeces (38 %). Of the radioactivity eliminated via these routes most was excreted during the first 48 h after dosing (91 % of the total recovery in the urine and 67 % of that recovered in the faeces). However, faecal elimination continued throughout the study with similar amounts recovered via this route during the 72-96 h period as to that recovered during the period 24-48 h post-dose. The Authors indicate that some of the marmosets did not defecate regularly leading to probable retention in the carcass and the variation and extension in elimination via this route. A small amount (2.3 %) of the administered radioactivity was recovered in the expired air as $^{14}CO_2$, again the majority (83 %) of this within the first 48 h after dosing. The radioactivity remaining in the carcass accounted for around 7 % of the administered dose; as indicated above, this level may have been influenced by the presence of faeces in the GI tract at the end of the study. Analysis of urine and faeces for metabolites revealed the presence of ETU, EU and unidentified polar material. ETU in the urine was found to account for ~19 % of the administered radioactivity, EU for 2 % and the polar material for ~11 %. In faeces, ETU accounted for ~2 % of the administered radioactivity, EU for 0.4% and the polar material for 2.5 %. However, not all of the radioactive material was extractable from the excreta, with only about two-thirds recoverable from urine, one-third from faeces and thus around 50 % overall. Thus the amounts of metabolites present may have been underestimated. The pattern of excretion of the metabolites that were extracted was similar to the overall pattern of excretion in that most was excreted within the first 48 h

via both routes. As an interesting extension to this work, the Authors demonstrated, through a repeat experiment of the marmoset study that ETU was photolabile (~7 % less recovered) in the presence of excreta, probably leading to the formation of polar material that was increased (by about 10 %). However, protection from light exposure was ensured in the main study.

3.2.1.1.2 Mancozeb

3.2.1.1.2.1 Rat

An extensive unpublished study is available in which the toxicokinetics of mancozeb was investigated following oral dosing. Radiolabelled mancozeb was synthesised for the studies with ^{14}C situated in the ethylene moiety. Sprague-Dawley rats were used for all of the studies that were performed to GLP. Throughout these studies radioactive mancozeb was administered by gavage as a suspension in 0.5 % (w/v) methylcellulose in distilled water. It is not stated in the test report whether or not the animals were fasted prior to dosing. All values presented are group means unless otherwise stated (Unpublished, 1986b; Unpublished, 1986c).

In one study group of 17 male and 17 female rats received a single dose by gavage of either 1.5 or 100 mg kg^{-1} ^{14}C-mancozeb. Sino-orbital blood samples were obtained from three rats of each sex at each dose at 0.5 h after dosing. These groups were then killed 30 minutes later and whole blood, liver and thyroid taken. Blood was sampled from another three rats from each sex and dose group at 3 h after dosing and these were killed at 6 h with the same tissues taken. At 24 and 48 h after dosing groups of three rats of each sex from each dose were killed and the same tissues sampled. The final group of five animals of each sex at both doses were used to carry out a balance study at 96 h. In these groups urine and faeces were collected over the whole experimental period (sampling periods of 0-6, 6-24 and every 24 h thereafter). At 96 h all animals were killed and the following tissues sampled: whole blood, plasma, liver, thyroid, adipose tissue, kidney, lung, heart, bone marrow, gonads, muscle, spleen and brain. For all the groups, radioactivity was assessed in all tissues (including whole blood and plasma) and excreta and ETU and EBDC measured in most cases in the plasma, liver and thyroid.

Recovery of radiolabel in the balance part of the study was essentially complete (92-124 % of the administered dose). Most of the radioactivity (80-95%) was recovered in the urine and faeces in both sexes at both doses after 96 h. Urinary excretion accounted for around half (49-54 %) of the administered dose with slightly more (46-65 %) eliminated via the faeces. Small amounts (1-2%) of radioactivity were retained in tissues with the remainder being found in the cage wash. Elimination of radioactivity was relatively rapid with most (80-90 %) of the radioactivity being excreted in the urine and faeces within the first 24 h after dosing. Uptake of radiolabel into blood was rapid in both sexes at both dose levels, though slightly quicker at the lower dose. Peak plasma concentrations were achieved within 3 h after 1.5 mg kg^{-1} compared to a peak at 6 h after 100 mg kg^{-1}. Elimination from plasma was biphasic with an initial rapid removal (lower dose $t_{1/2}$ of ~4 h compared to 6 h at the higher dose) and then slower phases ($t_{1/2}$ of ~23 and ~39 h respectively). Essentially similar results were obtained for whole blood, though the second elimination phase was longer than in plasma. Radioactivity in the liver reached a peak at 6 h after dosing in all groups expect for low dose females that showed a peak at 1 h. Elimination of radioactivity from the liver was also biphasic with a rapid phase of 6-8 h and a slower one of 31-38 h. Radioactivity in the

thyroid was also assessed over the study period. Peak levels were achieved after 6 and 24 h in the low and high dose groups respectively (no difference between sexes). The radioactivity in the thyroid then decreased between 24 and 48 h and then either remained at the same level or increased again (in the high dose group) to the end of the study. Peak radioactivity concentrations in the thyroid were found to be ~40-45 fold higher than whole blood at the lower dose and ~6-15-fold higher at the high dose. As indicated above, tissue distribution studies at 96 h after dosing indicated that only a small amount of the administered radioactivity was retained in body tissue. In general, for each group and tissue the retained dose was comparable between males and females except for adipose tissue and gonads where female concentrations were 2-11 fold higher than those in males. As a percentage of the dose administered, muscle contained the greatest amounts (0.42 - 1 %) with the thyroid containing 0.03-0.1 % and the liver 0.1-0.3 %. When expressed as a tissue concentration (in ppm), the thyroid contained the most (4-6 ppm at the lower dose; 79-131 ppm at the high dose) radioactivity these levels being ~10-19 fold greater than those in the next greatest tissue concentrations in the liver, kidney and spleen.

EBDC was not detected in the liver of rats of either sex at the lower dose at either 1 or 6 h after dosing. Some EBDC was found in the liver of high dose rats in both sexes up to 24 h after dosing. At 6 h after dosing EBDC levels were estimated to be ~190-290-fold less than the peak radioactivity observed at this time, indicating substantial metabolism of the parent compound. EBDC was not detected in the thyroid of high dose rats (and thus would probably not have been found in low dose rats) 24 h after dosing. ETU concentrations in plasma and liver of rats 6 h after a dose of 1.5 mg kg^{-1} ^{14}C-mancozeb were 5-7 and ~12-fold lower than the corresponding levels of ^{14}C. Furthermore, ETU was not detected in the thyroid of these animals at this time. In rats which received 100 mg kg^{-1} ^{14}C-mancozeb, the greatest plasma ETU concentrations were observed at 6 h after dosing and were ~6-13-fold less than the ^{14}C levels in both sexes. ETU was rapidly eliminated from the plasma and had decreased to below the level of detection 48 h after dosing. The AUC for plasma ETU concentration was 6.4 % and 3.1 % of the AUC for the ^{14}C curve for males and females respectively at the high dose, giving an indication of the bioavailability of ETU from administered mancozeb under the conditions of this study.

Liver ETU concentrations in rats dosed with 100 mg kg^{-1} ^{14}C-mancozeb were 95-107-fold lower than the corresponding liver ^{14}C concentrations in males and females at 6 h after dosing when the concentrations of both peaked in the liver. As with the plasma, hepatic ETU levels rapidly decreased and none was detectable at 48 h after dosing. In male rats dosed with 100 mg kg^{-1} ^{14}C-mancozeb, ETU was detected in the thyroid at only 6 and 24 h after dosing. At these times, the levels were 78 and 104-fold lower than the corresponding ^{14}C concentrations. In females at this dose, ETU was detected in the thyroid at 1, 6 and 24 h after dosing, the levels being 30-226-fold lower than the ^{14}C concentrations. Peak thyroid ETU concentrations were ~2-fold less than peak plasma concentrations in males and at similar peak levels in females.

In a preliminary range-finding study pairs of male rats received either 1.5 or 100 mg kg^{-1} ^{14}C-mancozeb. Excreta, including expired air, were collected up to 96 h and limited tissue samples were taken; blood was also sampled from 1 to 72 h. The results obtained were generally in line with those obtained in the main study. Only small amounts (0.3 and 1.3 % of the administered doses respectively) of radioactivity were detected in the expired air.

As an adjunct to these studies further groups of 5 male and 5 female rats received 15 ppm (stated to be equivalent to a dose of ~1.5 mg kg^{-1} d^{-1}) non-radiolabelled mancozeb in the diet for two weeks. At the end of this period each rat received a single oral gavage dose of 1.5 mg kg^{-1} ^{14}C-mancozeb. Excreta were collected as in the single dose study and the animals were killed at 96 h at which time the same selection of tissues were taken for determination of radioactivity, ETU and EBDC. The results obtained from this study were essentially similar to those following the single dose of 1.5 mg kg^{-1} ^{14}C-mancozeb indicating that dietary exposure at this level had no significant effect on the toxicokinetics of the substance.

As another part of the main study, groups of 3 rats of each sex with indwelling bile duct cannula received either 1.5 or 100 mg kg^{-1} ^{14}C-mancozeb. Bile was collected during the first 6 h after dosing and then from 6-24 h. Only radiolabel present in the bile was measured in these animals. The volume of bile collected in both sexes at both doses was similar. Radioactivity present in the bile over this time period accounted for 6-8% of the administered radioactivity in the low dose animals and 2-4 % in the high dose groups.

The profile of metabolites was also determined in the urine, faeces and bile of the rats from these studies. Urinary metabolites were characterized directly by TLC. Faeces were extracted and mancozeb (as EBDC) was assessed by HPLC and metabolites by TLC. The major urinary metabolite was ETU that accounted for 30-43 % of the radioactivity in both sexes at both dose levels. Since urinary excretion accounted for around half the administered dose, this would equate to at least 20 % being converted to ETU. Other urinary metabolites included EU (7-13 %), N-AcEDA (6-15 %), EDA (5-8 %) and EBIS (~1-2 %). A number of other derivatives were also detected which remained unidentified but none of these accounted for more than 10 % of urinary radioactivity. The major metabolite in the faeces was also ETU accounting for ~2.5-3 % of faecal radioactivity in the low dose group and 11-12 % in the high dose group. Other metabolites were also present including EU, N-AcEDA, EDA (all at 2-5 %) and EBIS (~1-2 %). Unmetabolised mancozeb was also detected at both dose levels but the amount found varied depending upon the detection method. By HPLC around 7-9 % unmetabolised mancozeb was detected, whereas 30-48 % was detected using the CS_2 method (based on the ability to generate CS_2 from EBDCs under acid conditions). The Author argued that this might have been due to degradation of mancozeb in faeces and/or binding to faeces making extraction for HPLC limited. ETU was also present in the bile, the amount being greater in high dose rats (11-15 % of the radioactivity) compared to the low dose (~4 %). Again the other identified metabolites were present in bile, accounting for ~3-8 % of the radioactivity in each case (the levels of EDA were significantly higher (11-32 %, thought to be due to the possible presence of glycine) (Unpublished, 1986d).

In summary, the results from this extensive series of investigations demonstrate that when administered by oral gavage to rats around 50-60 % of the organic portion of mancozeb is taken up rapidly into the body. Once absorbed, this is largely metabolised to a number of metabolites principally ETU, EDA, EU and N-AcEDA. Small amounts (1-2 %) of these metabolites and/or the organic portion of the parent molecule are widely distributed around the body with the muscle and thyroid particular sites for uptake. Most of the absorbed portion is eliminated from the body relatively rapidly within the first 24-48 h via the urine with small amounts excreted in exhaled air and the bile. Unabsorbed mancozeb is rapidly excreted in the faeces mostly within the first 24-48 h after dosing.

In an unpublished study, groups of Sprague-Dawley rats (14 per sex per group) were continuously administered mancozeb (a technical material, 84 % purity, remaining

composition not stated) in the diet at concentrations of 0, 30, 60, 125, 250 or 1,000 ppm for up to 3 months. The mean calculated doses of mancozeb received, as a function of body weight and corrected to the nearest whole number, were 0, 2, 4, 7, 15 or 57 mg kg^{-1} d^{-1} in males and 0, 2, 4, 9, 18 or 75 mg kg^{-1} d^{-1} in females. Ethylenebisdithiocarbamate (EBDC) and ETU levels were measured in the blood, urine and thyroid glands of 4 animals per sex per group, at study termination. No EBDC or ETU was detected in the blood of animals receiving up to and including 1,000 ppm (levels of detection were 0.05 and 0.1 ppm, respectively). In urine ETU levels increased in a dose-related fashion from 0.3 ppm at 30 ppm mancozeb to 10 ppm at 1,000 ppm mancozeb. EBDC levels of 0.1 to 1.1 ppm were measured in urine of animals at 125 to 1,000 ppm mancozeb. No EBDC was detected in the urine of animals at lower mancozeb exposure concentrations or in the thyroid glands of animals across the exposure range (limit of detection 25 ppm). However ETU levels of were detected in a dose-related manner from <4 ppm at 30 ppm mancozeb to 25 ppm at 1,000 ppm mancozeb (limit of detection was reported as 4 ppm) (Unpublished, 1986e).

3.2.1.1.2.2 Mouse

The toxicokinetics of mancozeb following oral dosing have been extensively investigated in the CD-1 mouse in an unpublished study conducted to US EPA guidelines and GLP. ^{14}C-mancozeb (labelled in the ethylene moiety) was obtained commercially and the purity confirmed prior to the investigation. It was administered as by oral gavage suspended in 1 % aqueous sodium carboxymethylcellulose in all of the investigations described below. All values presented are group means unless otherwise stated (Unpublished, 1990a).

In a single dose balance study groups of 5 male and 5 female mice received either 2.5 or 150 mg kg^{-1} ^{14}C-mancozeb by gavage and were then kept in glass metabolism cages until the end of the experiment. Urine and faeces were collected at 0-8, 8-24 h after exposure and then over 24 h intervals up to 168 h after exposure. From one mouse of each group expired air was collected and passed through traps designed to capture both CO_2 and CS_2 at 0-8 and 8-24 h after dosing at both doses and was also collected at 24-48 and 48-72 h after the higher dose. At the end of the study animals were killed and the radioactivity determined in a wide range of tissues, including plasma.

Recovery of radioactivity was essentially complete in all of the groups. Most of the administered radioactivity (86-95 %) was recovered in the urine and faeces at both doses with essentially little difference between sexes. For both doses and in both sexes, more radioactivity was eliminated via the faeces (48-64 % of the administered dose) than excreted via the urine (31-43 %). Most of the urinary (92-96 % of the radioactivity excreted via this route) and faecal (94-98% of the radioactivity excreted via this route) elimination occurred over the first 24 h after dosing. Radioactivity excreted via expired air as CO_2 accounted for ~0.4% of the dose at 2.5 mg kg^{-1} and ~4 % of the dose at 150 mg kg^{-1}. The presence of CS_2 was determined analytically and was found to be at the limit of detection in the low dose group. In the high dose group, it was estimated that over the 72 h collection period for expired air, the amount of CS_2 collected represented around 4 % of the administered dose. At the end of the study, the levels of radioactivity in whole blood and plasma were close to the limit of detection in the low dose groups. In the high dose groups, the levels of radioactivity were higher than at the lower dose and in whole blood compared to plasma. In general the level of radioactivity remaining in tissues at the end of the study was low, accounting for ~0.3 % of the administered low dose and ~1.3 % of the high dose. At both dose levels and in

both sexes, the highest levels of activity (expressed in tissue concentration terms) were found in the thyroid, liver and kidney.

In addition to the single dose study, a further group of 5 males and females received a daily gavage dose of 2.5 mg kg^{-1} unlabelled mancozeb for 14 d. On the 15th day they received a single 2.5 mg kg^{-1} dose of ^{14}C-mancozeb and underwent subsequent treatment as for the single dose balance groups. The results obtained for this group were essentially the same as for the single dose 2.5 mg kg^{-1} group, indicating that repeated exposure at this dose level had no significant effect on the toxicokinetics of mancozeb.

Limited metabolite profiling (to detect the presence of ETU only) was undertaken on the pooled urine from the 0-8 and 8-24 h collections from all of these (single and repeated dose) groups. (A separate more extensive investigation of the metabolic profile was conducted separately and is described below). Since most of the urinary excretion was completed during the first 24 h after the radioactive dose was administered these pools represented >90 % of the total radioactivity recovered via this route. Profiling was undertaken using 2-dimensional thin layer chromatography (2-D TLC) and the percentage of the radioactivity dose in the urine that had been converted to ETU was estimated. Following a single oral dose of 2.5 mg kg^{-1} ^{14}C-mancozeb (either as one dose only or when administered after repeated dosing with non-labelled mancozeb) ETU was estimated to represent 0.8-4.6% of the total urinary radioactivity. The higher figure of 4.6 % was found in single-dosed females; following multiple dosing the value was 1.7 % in females. The value obtained for ETU following the high dose was similar to the low dose at ~1 % in both sexes.

The blood kinetics of mancozeb were investigated as part of this study. Groups of 14 male and 14 female mice received a single gavage dose of 2.5 mg kg^{-1} ^{14}C-mancozeb. Two mice of each sex were killed and cardiac blood samples taken for the determination of radioactivity at each of the following times: 0.25, 0.5, 1, 2, 4, 8 and 24 h after dosing. In males, the peak blood levels of mancozeb were reached at 1 h after dosing and then fell slowly to about one-sixth of the peak level at 24 h. In females, the peak blood level was reached after 2 h and again declined slowly to around one-seventh the peak level at 24 h.

A quantitative tissue distribution study was also carried out in groups of 12 male and 12 female mice that received 2.5 mg kg^{-1} ^{14}C-mancozeb as a single gavage dose. Three mice of each sex were killed at 1, 8, 24 and 48 h after dosing. The radioactivity in body fluids and tissues was determined at each of these times. A qualitative study was also performed in which 2 male and 2 female mice also received 2.5 mg kg^{-1} ^{14}C-mancozeb as a single dose. One of each sex was killed at 1 h and 48 h after dosing and sagittal sections prepared for whole-body autoradiography. Data from the quantitative study showed that mean levels of radioactivity in organs and tissues peaked at around 1 h post dose and thereafter declined to levels close to or at the limit of detection by 24 to 48 h. The highest levels of radioactivity at 1 h after dosing were associated with the GI tract. Levels in the liver and kidney at this time were approximately an order of magnitude lower. Autoradiographic analysis revealed that at 1 h after dosing radioactivity was present in all tissues, the highest intensity being associated with the GI tract and major excretory organs. At 48 h post-dosing the levels of radioactivity recorded on the autoradiographs were low.

Further to all of the above studies, biliary excretion of ^{14}C-mancozeb was also studied in groups of 4 male and 4 female mice following a single dose of 2.5 mg kg^{-1} ^{14}C-mancozeb. One mouse of each sex was killed at 1, 8, 24 and 48 h after dosing. Immediately after being

killed the entire gall bladder was removed and the radioactivity present within determined. Excreta were collected every 24 h or at the time the animals were killed. Peak concentrations of radioactivity in the gall bladder were found at 1 h after dosing, accounting for 0.06 % (male) and 0.15 % (female) of the administered dose. At 8 h the proportion of the administered dose found in the gall bladder was 0.03 % (male) and 0.02 % (female), with no radioactivity being found at the subsequent time points.

As indicated above, in addition to all of these studies, the metabolic profile of ^{14}C-mancozeb was investigated in a further independent study. The in-life part of this was carried out in the same laboratories as all of the above studies using the same methodologies. The analytical analyses for the determination of metabolites in excreta were performed in a different laboratory, although again under conditions of GLP. Essentially repeat experiments of the balance studies were performed on groups of 5 male and 5 female mice that received either the single 2.5 or 150 mg kg-1 ^{14}C-mancozeb or the repeat 2.5 mg kg^{-1} dose of unlabelled mancozeb for 14 d followed by a single dose of 2.5 mg kg^{-1} ^{14}C-mancozeb. Urine and faeces were collected over the 24 h post-dose period as before and frozen for the subsequent analysis (Unpublished, 1992a).

The proportion of the radioactive dose recovered in each group was found to be 24-33 % in urine for the single dose studies in both male and females; 61-68% in faeces in males and 41-49 % in females for both single doses. In the repeat dose study, 50 % and 40 % radioactivity was found in the urine of males and females respectively but only 13% and 9% respectively was found in the faeces. Metabolites were identified using 2-D TLC. Urinary and faecal metabolites (expressed as percent of the administered dose) were: ETU (3-9 % in all cases except for 17 % in single high dose male faeces), EBIS/Ethylene thiourea-N-thiocarbamide (3-9 % in all cases except for ~0.4% in the faeces of repeat dose animals), NAcEDA (1-3 % in urine; 10 % in urine of repeatedly dosed males; generally not detected in faeces), EDA (1-5 % in urine and faeces), EU (2-7 % in urine and faeces), creatine (~0.5-2 % in urine only) and allantoin (~0.2-1 % only in urine). Six further unknown metabolites were also observed, occurring at 1-9 % of radioactive dose; one was tentatively identified as Jaffe's base.

3.2.1.1.2.3 Dog

In a study conducted according to modern protocol standards, in accordance with GLP, groups of 6 male and 6 female beagles received 0, 10, 100, 1000, or 5000 ppm mancozeb (technical grade material, 83 % pure, other components not stated) by dietary administration for 13 weeks. The mean intakes of mancozeb were determined to be approximately 0, 0.3, 3, 29, and 102 mg kg^{-1} d^{-1} for males and 0, 0.3, 3, 29, and 109 mg kg^{-1} d^{-1} for females. In addition to investigations of toxicity (see Section 3.3.4.1.1.2.2) the levels of ETU and EBDC were measured in the thyroid and urine at 13 weeks. EBDC and ETU were detected in the urine, and blood in a dose-dependent manner. ETU, but not EBDC was detected in the thyroid also in a dose-dependent manner. At dietary intakes of 10, 100, 1000, and 5000 ppm mancozeb, blood samples from males showed levels of ETU to be around 0.06 ppm, 0.07 ppm, 0.05 ppm, and 1.9 ppm, respectively, and for females, blood levels of ETU were around 0.04 ppm (limit of detection was 0.04 ppm), 0.07 ppm, and 0.19 ppm, respectively. In the thyroids, at dietary intakes of 10, 100, 1000, and 5000 ppm mancozeb, levels of ETU for males were <0.5 ppm, about 4 ppm, 9.9 ppm, and 18 ppm respectively, and for females, levels were <0.7 ppm, around 0.9 ppm, 9.2 ppm, and 15 ppm, respectively. In 24 h urine samples, levels of ETU for males were about 0.27 ppm, 1.7 ppm, 32 ppm, and 150 ppm respectively at 10, 100, 1000, and 5000 ppm mancozeb and for females, levels were about 0.2

ppm, 1.5 ppm, 2.1 ppm, and 93 ppm respectively at 10, 100, 1000, and 5000 ppm mancozeb. The mean total amount of EBDC in urine collected over 24 h for males was <0.24 μg, 24 μg, 247 μg, and 1261 μg at 10, 100, 1000, and 5000 ppm mancozeb; and for females was <0.12 μg, 12 μg, 149 μg, and 741 μg at 10, 100, 1000, and 5000 ppm mancozeb. Overall, the results indicate that mancozeb was absorbed following oral (dietary) administration, and potential metabolites EBDC and ETU were detectable in the thyroid with the urine identified as at least one of the routes of elimination (Unpublished, 1986f).

3.2.1.1.2.4 Goat

An unpublished study investigated that toxicokinetics of ^{14}C-mancozeb in lactating dairy goats. ^{14}C-mancozeb (labelled in the ethylene moiety) was administered by gelatin capsule daily for 7 d to 5 lactating goats at levels of 0.15, 0.7, 0.77, 1.7 or 1.8 mg kg^{-1} d^{-1}. Urine and faeces were collected daily during the study. Animals were milked morning and afternoon, and composite samples from these milking used for analyses. The goats were killed one day after the last dose (one of the high dose animals was killed 7 d after the last dose but results obtained were essentially similar to those from the other animals). A range of tissues (blood, fat, kidneys, liver, heart, gall bladder contents, thyroid) was taken for analysis of radioactivity (total and tissue component distribution) and in some cases metabolite content. Overall recovery of dosed radioactivity was 79-85 %. Most of the radioactivity recovered was found in the faeces (41-50 %) and urine (31-37 %). Of the body tissues, the liver and muscle was found to contain most radioactivity (1-2 % of that administered), with other tissues generally containing <1 %. Some radioactivity was found in milk, 0.17 % of that administered in the low dose animal to 1.5 % of that to the high dose animals. Separation of the milk revealed that the radioactivity was apportioned as follows: 23-30 % associated with protein; 5-17 % with lipid; 6-10 % in lactose (carbohydrate); and 43-60 % in the soluble fraction. Analysis (by TLC) of the radioactivity in the soluble fraction revealed it to be associated with the metabolites of mancozeb, namely EDA, N-AcEDA, ETU and EU. These products were also found in other tissues accounting for ~1 % of the total radioactivity recovered. Analysis of muscle and liver samples revealed that a significant portion of the radioactivity found in these tissues was associated with proteins, glycogen, creatines and lipids. Overall, this study demonstrates that a small proportion of the organic portion of mancozeb was excreted in the breast milk of goats and retained in tissue. Incorporation into the body carbon pools accounted for some of the retained radioactivity, whilst small amounts are found as metabolites (Unpublished, 1986g and 1986o).

3.2.1.2 DERMAL

3.2.1.2.1 Zineb

A well-conducted dermal exposure balance study is described in an unpublished report. For these studies, ^{14}C-zineb was synthesised as for the oral toxicokinetic studies, performed by the same workers, described above. Groups of 24 male Sprague-Dawley derived rats were used at each of four dose levels studied; 0.24, 2.4, 24 and 240 mg kg^{-1}. These dose levels were estimated to correspond to 0.004, 0.04, 0.4 and 4 $mg.cm^{-2}$. ^{14}C-zineb was applied to a shaved area (~13 cm^{-2}) of the back of each rat as a suspension in an aqueous medium for up to 8 h. The application area was covered by, but did not make contact with, a protective nylon mesh during the exposure period. Four rats from each dose group were killed at 1, 2, 4, 8, 24 and 96 h after the application of the zineb. Urine and faeces were collected until the time that the rats were killed in the 1, 2, 4 and 8 h groups; for 0-8 and 8-24 h in those killed at

24 and 96 h and also during every subsequent 24 h period for the 96 h group. At 8 h the application area in the rats killed at 8, 24 and 96 h was swabbed for the detection of residual activity and in the 24 and 96 h rats the area then covered in surgical gauze. At sacrifice, the kidneys, thyroid, liver, GI tract, blood samples and the area of treated skin were obtained and assessed for radioactivity. Data presented represent group mean values unless stated otherwise.

Generally most (>90 % and in many cases 100 %) of the applied radioactivity was recovered from the animals at all doses over all time points (slightly less - 87 % - was recovered at the lowest dose after 1 and 2 h). Although there was some variation at all dose levels over all time points the total amount of radioactivity absorbed accounted for <1 % of the applied dose. Values varied between the lowest proportion absorbed of <0.02 % of the applied dose to the highest at 0.68 %; up to 24 mg kg^{-1} there were no clear relationships between the amount absorbed and the dose or duration of exposure. The proportion of radioactivity absorbed at 240 mg kg^{-1} was generally slightly higher than at the other doses, though still within the range indicated. The radioactivity that was not absorbed was recovered from the skin. It is unclear if any of the radioactivity was retained in the skin itself, though most was recovered from the skin washes at 8 h suggesting that this was unlikely. Of the radioactivity that was taken up, most was eliminated via the urine with small amounts present in the faeces and carcass. Only very small amounts of radioactivity were found to be distributed around the body (in those tissues examined), in general accounting for only 0.03 % or, in most cases, less of the applied dose. Overall, this study demonstrated that when zineb was applied to the skin of rats as a suspension in an aqueous medium for up to 8 h, very little was absorbed either into the skin or body (Unpublished, 1986h).

3.2.1.2.2 Mancozeb

3.2.1.2.2.1 Rat

A limited unpublished single dose balance study has been performed following dermal administration of mancozeb to rats. The study was performed under conditions of GLP and quality controlled. Commercially available mancozeb (80.6 % active ingredient, other components not specified) was suspended in distilled water and applied to the backs of groups of 12 males Crl:CD®DR rats at dose levels of 25 or 250 µg cm^{-2} active ingredient under a non-occlusive dressing. Four rats from each dose group were killed at 0, 10 or 24 h after application. At the end of the exposure period, the application site was wiped clean with a solution of aqueous 2 % $ZnCl_2$/2 % soap. The urine, faeces, cage wash, non-occlusive application site cover, application site wash and skin and remaining carcass were all analysed for mancozeb and ETU. A sample of dosing solution was also subject to the same analysis. Because of problems with the analytical procedures (see below), a repeat study at 25 µg cm^{-2} was performed.

The analytical technique for mancozeb chosen by the investigators depended upon the evolution of CS_2 from mancozeb. Unfortunately, considerable interference was found in all samples of tissue and excreta, excluding the wash-off samples. Similar interference was found even in samples from untreated animals. The investigators were unable to resolve the problems. Consequently, analysis of mancozeb was not possible. Similarly, interference was also found to occur with measurement of ETU. Overall, the Authors concluded that the only way to assess uptake was by comparison of wash-off levels of mancozeb with those measured

in the dosing solutions. Analysis of the wash-off material indicated that at the low dose 101, 99 and 97 % of the measured applied mancozeb was present in the washings at 0, 10 and 24 h after dosing respectively. At the high dose, these values were 94, 94 and 95 % respectively. Overall, therefore, it was estimated that at 25 μg cm^{-2} ~2 and 4 % of the administered dose was absorbed dermally at 10 and 24 h. For the high dose, the Authors argued that the mancozeb not present in the wash-off had been retained either at the skin surface or was bound within the skin. Given the problems with the analytical techniques and the fact that some uptake was observed at the lower dose then it would seem more reasonable to conclude that at least some of the mancozeb had been taken up, though probably only a small amount (Unpublished, 1988a).

3.2.1.2.2.2 Rabbit

In an unpublished study conducted in accordance with OECD guidelines, conforming to GLP, groups of 5 male and 5 female New Zealand White rabbits received 0, 62.5, 250, or 1000 mg kg^{-1} d^{-1} mancozeb technical (84 % pure; other components not stated) moistened with distilled water. The study was primarily designed to investigate repeat dose toxicity and further details are given in Section 3.3.4.1.2.2.3. The substance was applied repeatedly to shaved dorsal skin under an occlusive dressing for 6 h d^{-1} for 21 d by the dermal route; the application site was carefully washed and dried at the end of each 6 h application period. Blood samples were collected prior to termination and pooled to determine the presence of mancozeb and ETU in blood.

The analysis of blood samples for mancozeb and ETU indicated approximately 0.44 ppm mancozeb and 0.3 ppm ETU in males that had received 1000 mg kg^{-1} d^{-1}. For the remaining groups of males, the levels of mancozeb and ETU were below the level of detection (0.1 ppm) except for males at 250 mg kg^{-1} d^{-1} where 0.13 ppm mancozeb could be detected. For females at 1000 mg kg^{-1} d^{-1} 0.32 ppm mancozeb and 0.13 ppm ETU were found. As with the males, for the remaining groups, the levels of mancozeb and ETU were below the level of detection (0.1 ppm) except for females at 250 mg kg^{-1} d^{-1} where 0.13 ppm mancozeb could be detected. Overall, there was evidence that mancozeb was dermally absorbed, with some proportion being metabolised to ETU (Unpublished, 1988b).

3.2.1.3 INHALATION

3.2.1.3.1 Zineb

No inhalation toxicokinetic studies are available for zineb. Qualitative information from a repeated exposure inhalation study (see Section 3.3.4.3.3.1) suggests that zineb, and/or its metabolites, is taken up from the respiratory tract and distributed around the body at least to the liver and kidneys.

3.2.1.3.2 Mancozeb

In a study conducted in accordance with GLP, groups of 16 male and 16 female Sprague-Dawley rats were exposed, nose-only to 0, 8, 40, and 127 mg m^{-3} mancozeb (commercial technical material, 83% pure, other components not stated) for 6 h d^{-1}, 5 d $week^{-1}$ for 13 weeks followed by a 13-week recovery period. The mass median aerodynamic diameter was determined to be 3.8-4.2 μm, GSD 2.1. In addition to toxicity evaluations (see Section 3.3.7.1.3), ethylenethiourea (ETU) and ethylene bisdithiocarbamate (EBDC) levels were

measured in samples of blood and urine and ETU levels in the thyroid at week 13 and also at the end of the recovery period. Detectable levels of ETU were recorded in the blood of all mancozeb-exposed animals (0.12, 0.18, and 0.14 ppm amongst mancozeb-exposed exposed males respectively, 0.1, 0.17, 0.45 ppm amongst females). EBDC could only be detected in the blood of animals at the highest exposure level (males, 0.86 ppm, females, 0.91 ppm). ETU and EBDC were detected in the urine of all mancozeb-exposed animals (males 0.12, 0.66, 0.53 ppm, females 0.29, 1.3, 3.1 ppm). ETU was detected in the thyroids of males and females at 40 and 127 mg m^{-3} (males 5.1, 7.7 ppm, females 11.0, 28.0 ppm). At the end of the 13-week recovery period ETU and EBDC were not detected in blood, urine, or thyroid samples. Overall, this report indicates that mancozeb was absorbed to an indeterminate extent, by the inhalation route, metabolised at least to some extent to ETU and EBDC, that the metabolite ETU was distributed to the thyroid, and that the metabolites ETU and EBDC were eliminated in urine (Unpublished, 1986i).

3.2.2 STUDIES IN HUMANS

No data were submitted and no useful data were found in the literature. ETU was identified in the urine of workers who were potentially exposed to EBDCs (not further specified, although mancozeb is likely to have been used), however, no useful quantitative information is available (Steenland *et al*, 1997). Further methodological details of this study are given in Section 3.3.4.5.

3.2.3 SUMMARY OF TOXICOKINETICS

No useful data are available on the toxicokinetics of zineb in humans. There is a variable amount of information available from studies in experimental animals, depending upon the route of exposure. Furthermore, of those studies that are available, none have investigated the fate of the zinc ion and all have focused on the toxicokinetics of the organic portion of the molecule. Although the fate of the zinc from the molecule is unclear, the toxicology profile of zineb does not appear to indicate any significant involvement of zinc in its toxicity.

The toxicokinetics of the organic part of zineb following oral dosing has been well studied in rats, though over a relatively small dose range, with limited studies in the mouse and marmoset. Following gavage dosing (5 or 50 mg kg^{-1}) in the rat around 50-70 % of the administered dose is taken up rapidly across the GI tract. An older dietary study in rats has suggested a lower (up to 20 %) uptake but these data should be viewed with some caution because of limitations in the design and reporting of the study. Oral gavage dosing of marmosets has also indicated that at least 50-60 % of the administered dose is taken up rapidly in this species. The only study in the mouse indicated uptake to be low at ~6 % but as recovery of the administered dose was significantly incomplete this value should be treated with caution. Overall, uptake following oral dosing in the rat and marmoset is judged to be moderate and rapid. Distribution data are only available for the rat. Once absorbed, in rats the organic portion of the parent molecule and/or its metabolites are widely distributed throughout the body, with the muscle, liver, kidney, adrenal and thyroid all being particular sites of uptake (in terms of tissue concentration the thyroid is the most significant organ). With respect to metabolism, the available information indicates that in all species studied zineb is metabolised to ETU. The most extensive studies are for the rat, in which it has been shown that at least 20% of an administered oral dose is converted to ETU, with the presence of the ETU metabolites EU and TU found in significant quantities (4-15 % of an administered dose). EDA (~10 % of a dose) has also been found to be produced from zineb

as has CS_2 qualitatively. Studies in the marmoset indicate that at least 20 % of an administered dose is converted to ETU in this species. It is difficult to define a value for the mouse as recovery was incomplete. A proposed metabolic pathway for zineb following oral dosing is shown at Figure 3.1. Following oral dosing, the organic portion of zineb is excreted relatively rapidly in all species studied, mainly via the urine and in the faeces. Urinary excretion is largely in the form of metabolites. Faecal content is mainly unabsorbed zineb in rats since biliary excretion accounts for only a small amount (1-6 %) of an administered dose. Small amounts, a few percent at most, of an administered dose have been found to be excreted in expired air as CO_2. Only relatively small amounts (1-2 %) have been found to be retained in the body in the rat and marmoset (incomplete recovery in the mouse makes it difficult to judge retention) following a single oral dose. There is some limited evidence from the rat for some bioaccumulation following repeated daily dosing, although the toxicokinetics otherwise were essentially similar as those for single dosing.

A similar oral toxicokinetics database is available for the organic portion of the structurally-related mancozeb with extensive studies in the rat and mouse and some limited information from the dog and lactating goat. No information was available on the fate of the zinc or manganese though as for zineb toxicodynamic data suggest that these do not play a significant role in toxicity. In general the toxicokinetics of mancozeb following oral dosing is broadly similar to that of zineb. Absorption across the GI tract appears to be similar with around 50-60 % of the organic portion being taken up rapidly in rats. Slightly less (up to 45 %) but rapid uptake was seen in the mouse and, based on limited information, around 50 % in lactating goats. Distribution of the absorbed portion of mancozeb and/or its metabolites is largely similar to that of zineb in that it is widespread, with the muscle, thyroid and liver being particular tissues for uptake in the rat. In the mouse, the evidence available did not indicate that uptake into the thyroid was more significant than other tissues. Studies in the goat indicated that some (possibly up to half) of the available carbon is recruited into general body pools. The metabolic profile of mancozeb is also similar to that of zineb in the rat with ETU again being a significant metabolite accounting for the conversion of at least 20 % of an administered dose. In contrast, ETU would appear to be a relatively minor metabolite of mancozeb in the mouse. A proposed metabolic pathway for mancozeb following oral dosing is shown at Figure 3.2. The excretion of mancozeb, like zineb, is rapid and principally via the faeces and urine, with small amounts excreted in expired air as CO_2 and CS_2. Evidence from the lactating goat indicates that a small proportion of the organic portion of mancozeb and/or its metabolites is excreted via the breast milk. Given many of the similarities of the toxicokinetic properties of zineb and mancozeb, it would seem reasonable to conclude that a small amount of zineb and/or its metabolites would also be available to breast milk.

The information available for the toxicokinetics of zineb following dermal dosing is limited to one good quality study in the rat. This indicated that over a wide dose range covering three orders of magnitude (expressed relative to body weight or surface area) that relatively little of the applied dose (<1 %) was absorbed into the body. Although of poor quality, a dermal study in rats with mancozeb generally confirmed the low bioavailability via this route. Mancozeb and ETU were detected in the blood of rabbits repeatedly exposed to mancozeb for 21 d. However, it is not possible to quantitate the extent of uptake directly from this study.

There are no specific studies on the toxicokinetics of zineb following inhalation exposure. Qualitative information from a limited repeat inhalation exposure toxicity study in rats with solid zineb powder suggests that uptake and distribution of the substance and/or its

metabolites may occur from the respiratory tract as indicated by toxicity in the liver and kidneys. This view is supported by the detection of EBDC and ETU in the blood, urine and thyroid of rats repeatedly exposed by inhalation to the structurally related mancozeb. The presence of a typical response to the presence of a dust in the respiratory tract of the zineb-exposed rats suggests that uptake may be limited via this route. Overall, though, the data available do not allow any quantitative conclusions to be drawn with respect to the toxicokinetics of zineb following inhalation exposure.

No specific information is available on whether or not zineb can cross the placenta and be taken up by developing offspring. It is known that the metabolite ETU (see Section 3.3.7.1.3**)** is able to reach the offspring as evidenced by effects on development. It would therefore be reasonable to assume that ETU generated following the metabolism of zineb may also be able to reach the developing offspring.

Overall, on considering the data available on the relative toxicokinetics of the organic portions of zineb and mancozeb, it would appear that on the whole the two substances are handled, at least in the rat, in a generally similar manner (both qualitatively and quantitatively). It would therefore appear reasonable to use toxicity data for mancozeb to read across to zineb where it is believed that any effects seen reflect a similar metabolite-related aetiology.

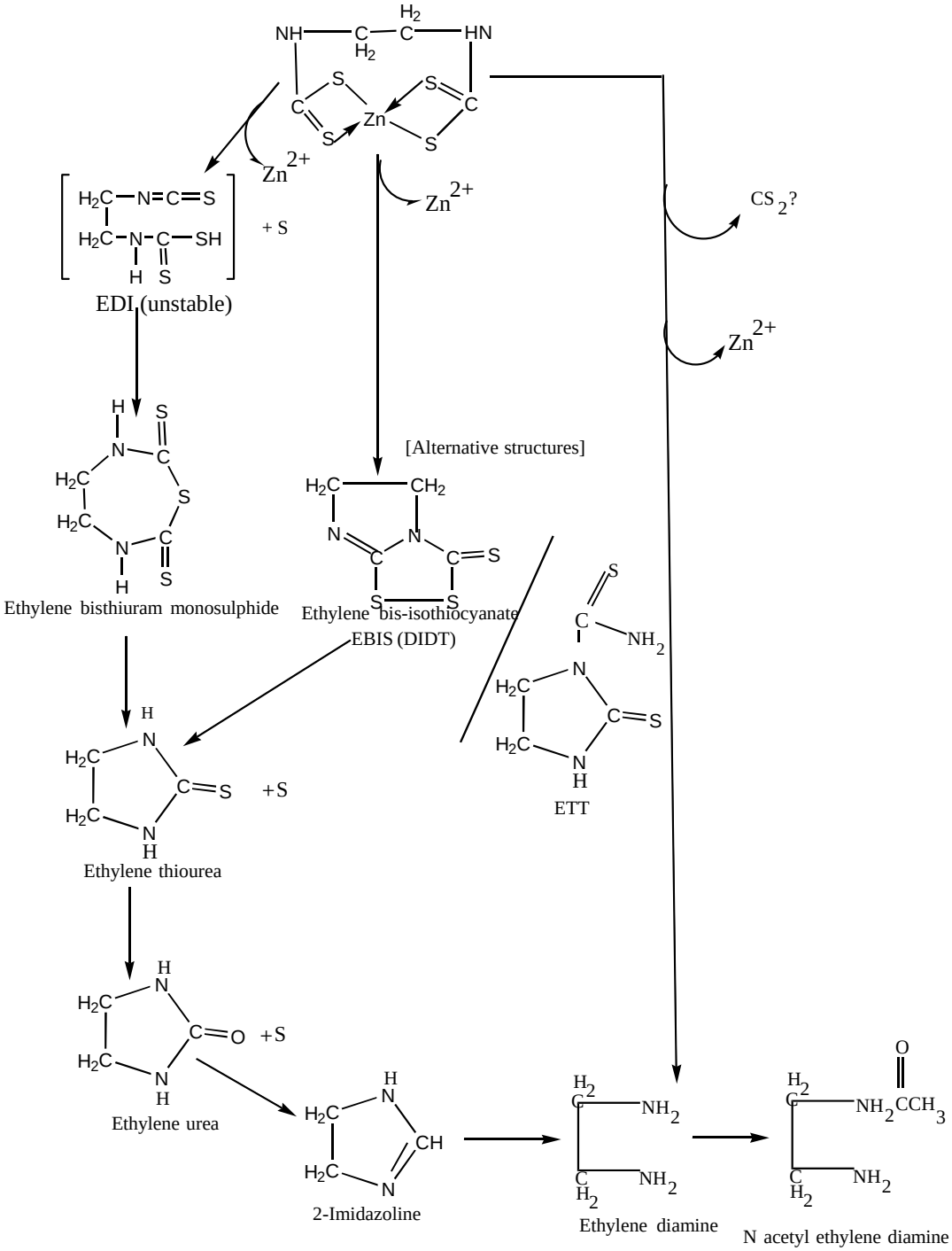


Figure 3.1 Proposed Metabolic Pathway for zineb

There is no information available regarding the chemical/enzymatic processes or of the specific enzymes involved in metabolism. Pathways are based on identified metabolites.

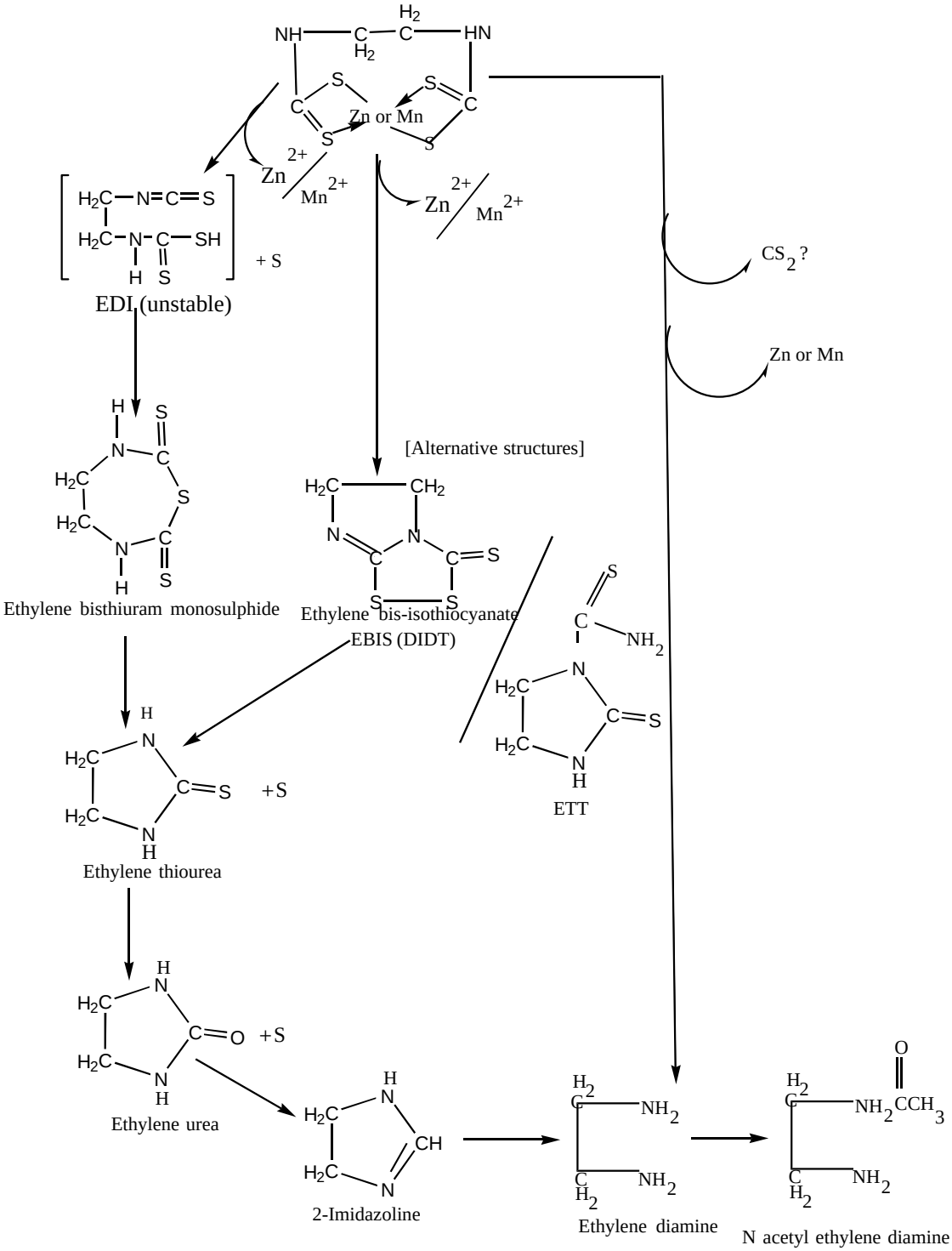


Figure 3.2. Proposed Metabolic Pathway for mancozeb

There is no information available regarding the chemical/enzymatic processes or of the specific enzymes involved in metabolism.

3.3 MAMMALIAN TOXICOLOGY

3.3.1 ACUTE TOXICITY

3.3.1.1 STUDIES IN ANIMALS

There are a number of published and unpublished reports available on the acute toxicity of zineb. In many cases the reports are limited in nature and generally there are relatively few details regarding the exact composition and purity of the material tested. However despite these deficiencies a generally consistent picture emerges from the data that are available.

3.3.1.1.1 Oral

3.3.1.1.1.1 Zineb

The most recent of the published studies available briefly describes in limited detail acute toxicity studies on zineb in rats and mice, conducted as part of a more extensive investigation on a number of fungicides. The zineb used in the studies, obtained commercially, was stated to contain 80 % active ingredient with the remaining constituents being 'inert material added by the manufacturers to make dry powders for spray suspensions'. Between 8 and 16 rats (CD) and mice (CD-1) of both sexes were used. The dose levels used and the number of animals/dose was not stated. Zineb was administered as a single gavage dose as a suspension in 0.5 % aqueous carboxymethylcellulose. The animals were fasted overnight before dosing and observed for 28 d afterwards. The only quantitative data given in the report are calculated LD_{50} values which were 8200 and 8900 mg kg^{-1} for male and female rats respectively and 7600 and 7700 mg kg^{-1} for male and female mice respectively. Most deaths (cause not given) were said to occur within 1-2 d of dosing. It was also stated that 'these fungicides' caused ataxia, hyperactivity followed by inactivity, loss of muscular tone and alopecia after high doses; it is not clear which (if any) of these signs were seen following treatment with zineb (Lee *et al*, 1978).

In an older study, again with limited reporting, the acute toxicity of zineb was studied as part of a more general investigation of the toxic properties of the substance (Blackwell-Smith *et al*, 1953). The zineb used was described as a '65 % commercial wettable powder', although no further information was provided on the composition or purity. For the acute oral studies young male 'albino' rats (strain not specified) were fasted overnight and received single gavage doses of zineb as either a 10 % or 20 % suspension in water. Two animals per dose received 650, 1300 or 2600 mg kg^{-1}; 20 rats received 5200 mg kg^{-1}, stated as being the highest dose achievable. No deaths or signs of toxicity were seen following any dose.

The acute toxicity of zineb was studied in rats and described in a limited report (Ivanova *et al*, 1966). The material used was described as containing 60 % active ingredient but no other information was provided. The strain of rats used was not stated. Groups of rats (number per group not stated) received oral gavage doses (dose levels not stated) of 20 % zineb solution but the vehicle was not stated. The LD_{50} was reported to be 4400 mg kg^{-1} but no details of toxic signs including deaths were given.

Three studies follow where the concentration of zineb tested has not been clearly identified, however, these reports corroborate the findings of the studies described previously.

In the first of these studies, an unpublished summary report provides some limited details on an acute oral study performed in rats. The report is very brief and provides only limited information. Groups of Wistar rats (a total number of 10 of each sex were used) received a single dose, by gavage, of 'professional' grade zineb (purity and any contaminants not stated) as a 20 % suspension in distilled water. The rats were fasted for 6 h prior to dosing. The number and size of doses was not stated though the highest dose was 10,000 mg kg^{-1}. No deaths or signs of toxicity were observed (Unpublished, 1976a).

In the second of these studies, the assessment of the acute oral toxicity of a 70 % w/w zineb formulation in rats is described in an unpublished summary report. The report is very brief and provides only limited information. Groups of Wistar rats (a total number of 10 of each sex were used) received a single dose, by gavage, of a 70 % w/w zineb formulation (purity and any contaminants not stated) as a 20 % suspension in distilled water. The rats were fasted for 6 h prior to dosing. The number and size of doses was not stated though the highest dose was 10,000 mg kg^{-1}. No deaths or signs of toxicity were observed (Unpublished, 1976b). An apparently identical study, described in an equally brief summary report, was performed using an 80 % w/w zineb formulation with the same outcome; no deaths or signs of toxicity were seen up to a dose of 10,000 mg kg^{-1} (Unpublished, 1976c).

Thirdly, results for zineb are included in a report which summarises a large-scale study, conducted over a number of years, to provide information on the acute toxicity of pesticides (Gaines, 1969). There are relatively few details given in the report, such as the doses used, although from the tabulated data it would appear that only a single dose was employed. Sherman rats (10 of each sex at each dose level) received a single gavage dose of 'technical grade' (purity and contaminants not stated) zineb dissolved or suspended (unclear from the report) in peanut oil. No deaths were apparently observed and the LD_{50} was given as >5000 mg kg^{-1} for both sexes. No information was given on whether or not any signs of toxicity were observed.

A brief report describes the effect of a single relatively high dose of zineb on the uptake of ^{131}I into the thyroid of rats (Ivanonva *et al*, 1967). A group of 10 'albino' rats (strain not reported) received a single dose of 2400 mg kg^{-1} zineb (stated to be 70 % but no further details given) as a suspension in water by oral gavage. A further group of 10 rats served as controls. At 24 h after dosing the animals received an i.p. dose of ^{131}I in saline. The uptake of ^{131}I in to the thyroid was then determined 2 h after dosing with the radioactive tracer. Around 10-fold less ^{131}I was found in the thyroid of treated rats compared to the controls. No other measurements were made.

The effect of zineb treatment on hepatic xenobiotic metabolism has been studied following oral dosing (Siddiqui *et al*, 1991). Groups of 5-6 albino rats (strain not specified) received a single oral gavage dose of 100 or 250 mg kg^{-1} zineb. The zineb (apparently a commercial preparation) was stated to be 82 % pure but the content of the other 18 % was not given. It was administered as a suspension in 1 % gum acacia; controls received gum acacia alone. The rats were killed at 1 and 4 h after treatment and the effects on hepatic mixed function oxidase (MFO) evaluated by assessing the activity of aminopyrine N-demethylase, p-nitroanisole *O*-dealkylase, aniline hydroxylase and benzo(a)pyrene hydroxylase. No changes were seen in benzo(a)pyrene hydroxylase activity under any treatment conditions. There

were statistically significant reductions (up to ~50 %) at 4 h in the activity of the other enzymes which tended to be slightly greater at the higher dose; aniline hydroxylase was also affected at 1 h at both doses and p-nitroanisole *O*-dealkylase at 1 h after 250 mg kg^{-1}. In light of the fact that repeated dosing for four weeks at 250 mg kg^{-1} (see Section 3.3.4.1.1.1) did not result in any similar changes, the significance of these findings is unclear.

A similar study carried out in rabbits has been reported (Nebbia *et al*, 1993). A group of 4 New Zealand White rabbits, fasted overnight prior to treatment, received a single gavage dose of 300 mg kg^{-1} zineb suspended in methylcellulose; a group of 4 rabbits that received only methylcellulose served as controls. The zineb, of technical grade, was stated to be 92 % pure with <0.25 % ETU present. The animals were killed 24 h after dosing, the liver removed and weighed and measurements of a range hepatic parameters including the activities of xenobiotic metabolising enzymes (MFO and glutathione transferase and reductases) made. Zineb did not affect absolute or relative liver weight or microsomal or protein content. There was a marked decline in the content of both P450 and total haem. The rate of metabolism of aniline (a measure of aniline-4-hydroxylase activity) was reduced by about 70 % but there was no change in carboxylesterase or glutathione-related enzymes.

3.3.1.1.1.2 Zineb formulation ‘a’

An unpublished summary report containing limited details is available of an acute oral toxicity study on a formulation ‘a’ containing 3.3 % zineb. The study was conducted to contemporary OECD standards and subject to quality assurance. Formulation ‘a’ was administered as a single gavage dose to fasted Sprague-Dawley rats (5 of each sex). The formulation, a red liquid, was diluted in corn oil and administered as a limit dose of 5000 mg kg^{-1} (chosen on the basis of a sighting study), equivalent to about 165 mg kg^{-1} zineb. Two deaths occurred which were thought to be due to dosing errors. No other deaths were observed. Signs of toxicity were stated as hypokinesia, sedation, hunched appearance, piloerection, soiled coat, ataxia, haemodacryorrhoea, epistaxis, excess salivation and dyspnoea. However, the time of onset, duration, severity and numbers of animals affected is not stated. Overall, it was concluded that the oral LD_{50} of formulation ‘a’ was >5000 mg kg^{-1} (Unpublished, 1984a).

3.3.1.1.1.3 Mancozeb

An unpublished report is available on the acute toxicity of mancozeb when administered by the oral route. The study was performed to contemporary OECD guidelines and GLP. Groups of five male and five female CFY rats received a single 5000 mg kg^{-1} dose of mancozeb (technical grade of 88.2 % purity) by oral gavage as a suspension in 1 % methylcellulose and observed for 14 d. No deaths were observed with the only treatment-related sign being piloerection, shortly after dosing (Unpublished, 1986j).

The acute toxicity of mancozeb was investigated in a published study (Kackar *et al*, 1997a). Groups of 10 male Wistar rats received a single oral gavage dose of 9600, 12000, 15000 or 18750 mg kg^{-1} mancozeb suspended in peanut oil. A control group of 10 rats received peanut oil. The mancozeb was obtained commercially and described as '75 wettable powder', with no further details on purity or composition being given. Animals were observed for 15 d after dosing. No deaths or signs of toxicity were seen at 9600 mg kg^{-1}. Deaths occurred at the higher doses (2 at 12000 mg kg^{-1}, 5 at 15000 mg kg^{-1} and 8 at 18750 mg kg^{-1}). Signs of toxicity at these dose levels included dyspnoea, diarrhoea, salivation, nasal bleeding and hind

limb paralysis; the onset and duration of these signs was not given. An LD_{50} of 15000 mg kg^{-1} and a NOAEL of 9600 mg kg^{-1} was identified for mancozeb from this study.

3.3.1.1.1.4 Ethylene thiourea

No data were submitted on the acute toxicity of ETU. Information contained in reviews indicate that in contrast to zineb and mancozeb, ETU has moderate acute toxicity via the oral route with LD_{50} values in rat estimated between 545 and 1832 mg kg^{-1} (IPCS, 1988).

3.3.1.1.2 Dermal

3.3.1.1.2.1 Zineb

The assessment of the acute dermal toxicity of a 70 % w/w zineb formulation in rats is described in an unpublished summary report. The report is very brief and provides only limited information. A group of 5 male and 5 female Wistar rats was treated topically for 24 h with 7000 mg kg^{-1} of a 70 % w/w zineb formulation (purity and any contaminants not stated). The material was applied 'undiluted' to a shaved wetted area; the type of dressing applied (if any) was not stated. Measures were taken to prevent oral ingestion from the application site during the 24 h exposure period. No deaths or signs of toxicity were observed (Unpublished, 1976d). An apparently identical study, described in an equally brief summary report, was performed using an 80 % w/w zineb formulation with the same outcome; no deaths or signs of toxicity were observed up to a dose of 7,000 mg kg^{-1} (Unpublished, 1976e).

Results for zineb are included in a report which summarises a large-scale study, conducted over a number of years, to provide information on the acute toxicity of pesticides (Gaines, 1969). There are relatively few details in the report including the doses used, although from the tabulated data it would appear that only a single dose was employed. Sherman rats (10 of each sex at each dose level) received a single dermal application of 'technical grade' (purity and contaminants not stated) zineb as a 25 % suspension in propylene glycol. Apparently no restraints were used and residues were not removed following dosing. No deaths were apparently observed and the LD_{50} was given as >2500 mg kg^{-1} for both sexes. No information was given on whether or not any signs of toxicity were observed.

3.3.1.1.2.2 Zineb formulation ‘a’

An unpublished summary report containing limited details is available of an acute dermal toxicity study conducted to OECD guidelines on formulation ‘a’ containing 3.3 % zineb. Formulation ‘a’ was administered as a single dermal application to Sprague-Dawley rats (5 of each sex). The formulation, a red liquid, was administered as a limit dose of 2000 mg kg^{-1}, equivalent to about 165 mg kg^{-1} zineb, for 24 h. The material was applied on gauze that was stuck down with adhesive tape. No deaths or signs of toxicity were observed (Unpublished, 1984a).

3.3.1.1.2.3 Mancozeb

The acute dermal toxicity of mancozeb has been investigated in an unpublished study. Five male and five female New Zealand White rabbits received an application of 2000 mg kg^{-1}

mancozeb technical (stated to be 88.2 % pure - other components not stated) for 24 h. The material was moistened with distilled water and placed under an occlusive dressing. No deaths or signs of systemic toxicity were observed. Very slight (grade 1) erythema was seen in 4 males and 4 females up to 6 d after the application (Unpublished, 1986k).

3.3.1.1.2.4 Ethylene thiourea

No data were submitted. Information contained in a review (IPCS, 1988) indicates ETU to be of low acute toxicity via the dermal route.

3.3.1.1.3 Inhalation

3.3.1.1.3.1 Zineb

No published, good quality, reports were available.

A single unpublished report, containing limited detail, is available describing a study on the acute inhalation toxicity of an 80 % w/w zineb formulation. The purity of the test material was not stated in the report. Groups of 10 male and 10 female Wistar rats were exposed nose-only for 4 h to an aerosol of an 80 % w/w zineb formulation at what appears to have been a single exposure concentration. The amount of an 80 % w/w zineb formulation that was present in the aerosol is unclear, although it is stated that a 10 % concentration in water, the highest achievable, was used as the stock solution from which the test aerosol was generated. Given that the water solubility of zineb is relatively poor, then it is unclear what the 10 % value represented. The final atmospheric concentration of the aerosols was cited as 20 ml l^{-1} and that the droplets were <5 mm, though no data were provided to support this statement. No deaths were observed. Treatment-related signs of toxicity were seen during and for the first few hours after exposure and consisted of increased respiratory rate, apathy and disturbances in co-ordination and rough coat. At necropsy there were no macroscopic treatment-related findings (Unpublished, 1977).

3.3.1.1.3.2 Mancozeb

A single unpublished report is available of a study on the acute inhalation toxicity of mancozeb. Groups of five male and five female Wistar rats were exposed, whole body, to a respirable dust of mancozeb technical (stated to be 88.2 % pure - other components not stated) for 4 h. Exposure concentrations (measured) used were 3.79 and 4.76 mg l^{-1}; control groups of five males and five females were exposed to air. Analysis of particle size distribution indicated that around 70 % of the dust (expressed in terms of mass) at both exposure concentrations was ≤ 5.5 µm in aerodynamic diameter. Animals were observed for 14 d after exposure. One male rat died following 3.79 mg l^{-1} mancozeb and 2 males following 4.76 mg l^{-1}, all deaths occurring within the first 24-48 h after exposure; no deaths were observed in females. The LC_{50} was therefore > 4.76 mg l^{-1} for both sexes but there would appear to be a sex difference, with this value probably being close to the LC_{50} for male rats.

Treatment-related signs of toxicity seen during exposure at both exposure concentrations were typical of exposure to a mildly irritant dust and included reduced respiratory rate, hunched posture and excessive salivation. Immediately after exposure some rats at both

exposure concentrations had increased respiratory rate, gasping and rales and at the higher concentration were lethargic. Lethargy (in males), increased respiratory rate and rales persisted in both males and females up to the 12th day post-exposure. Body weight gain in the high dose males was affected with a loss of weight on the day following exposure and subsequent reduced weight gain. Body weight gain was also reduced in females at both exposure concentrations, with weight loss again seen at the higher dose level. The loss of body weight and reduced weight gain were associated with reduced food consumption in the first week after exposure, particularly notable in the high dose group females. The only macroscopic finding in the surviving rats was congestion in the lungs of one female exposed to 3.79 mg l^{-1}. The lungs of all decedent rats were all congested. Furthermore, in two animals, the larynx was blocked by, and the trachea lined with, a white solid material. Histopathological examination of these indicated marked mucosal necrosis of the larynx. No other histopathological changes were observed (Unpublished, 1987a).

3.3.1.1.3.3 Ethylene thiourea

No data submitted and none in the published literature.

3.3.1.1.4 Studies in Humans

A case report is available regarding a 42 year old male who was admitted unconscious to a medical centre with occasional tonic and clonic convulsions and signs of right hemisporesis (Israeli *et al*, 1983). Prior to admission, he had been spraying a cucumber crop with a mancozeb-zineb mixture. He had used the mixture twice, each time using PPE, and had experienced no health effects. On the second occasion he had increased the concentration of the mixture 10-fold (though no details are given of the amounts actually used). On the day after each spraying he had walked through the treated crops without PPE, and subsequently began to feel ill. On the second occasion he complained of weakness, headache, nausea and fatigue. He became disoriented and lost consciousness, where after he was admitted to the medical centre. He recovered spontaneously within a few days after admission. Although it is possible that the effects experienced by this individual were related to his exposure to the mancozeb-zineb mixture remaining on the cucumber crop, no firm conclusions can be drawn given the lack of objective information on the exposure conditions which he had experienced.

3.3.1.2 SUMMARY OF ACUTE TOXICITY

No useful human data are available. There are few good quality animal studies available which have investigated the acute toxicity of zineb. However, those that are available present a reasonably consistent picture. Zineb is of low acute toxicity by the oral route, with LD_{50} values in excess of the highest doses tested that are of the order of grammes per kilogram. Mancozeb, a structurally similar substance is also of low acute oral toxicity. From the limited information available, the acute toxicity of zineb via the dermal route appears to be low, which is consistent with the low oral toxicity and poor bioavailability via this route. The only available study on the acute inhalation toxicity of zineb is of too poor a quality to draw any conclusions. An inhalation study on mancozeb resulted in the expression of acute effects with deaths in male rats and signs of toxicity in both sexes at around 5 mg l^{-1} suggesting low acute inhalation toxicity. This appeared to be related to local effects in the respiratory tract.

Data are available to indicate formulation ‘a’ containing 3.3 % zineb, was of low acute toxicity following oral and dermal administration.

3.3.2 IRRITANCY

3.3.2.1 STUDIES IN ANIMALS

3.3.2.1.1 Skin

3.3.2.1.1.1 Zineb

A brief summary report is available of a skin irritation study using 'technical' zineb. Undiluted 'technical' zineb (500 mg - purity and composition not stated) was applied with occlusion to an area of ~100 cm^2 of moistened skin on the backs of 6 New Zealand White rabbits for 4 h. The skin of each animal was examined daily for 14 d for signs of irritation. No signs of skin irritation (erythema or odema) were observed (Unpublished, 1976h).

An unpublished summary report is available of a skin irritation study using an 80 % w/w zineb formulation. The report is brief and limited in detail. An 80 % w/w zineb formulation (500 mg – no further composition details provided) was applied to the moistened skin of three New Zealand White rabbits for 4 h. The material was applied under polythene covered by an elastic bandage to an area of ~100 cm^2; this is slightly larger than required under current guidelines. A second application was applied to skin on which longitudinal abrasions had been formed prior to the experiment. In both cases, no signs of skin irritation were observed (Unpublished, 1976f).

3.3.2.1.1.2 Zineb formulation 'a'

The dermal irritation potential of formulation 'a' containing 3.3 % zineb has been investigated in rabbits. Six (3 male, 3 female) New Zealand White rabbits were used for each of the formulations tested, formulation 'a' and formulation 'b'. The latter formulation, used as a control, was stated to be the same as formulation 'a' except that it did not contain zineb. 0.5 ml of each material (liquids) was applied under an occlusive dressing for 4 h; each rabbit was treated at two separate patches thus giving a total of 12 tested sites. Skin reactions were scored at 0.5, 24, 48 and 72 h. Erythema and oedema were seen with roughly equal intensity for both formulations (mean scores over the 12 sites for formulation 'a' were 0.66 for erythema, 1.08 for oedema; for formulation 'b' were 0.83 for erythema, 0.94 for oedema) (Unpublished, 1984a).

3.3.2.1.1.3 Mancozeb

The skin irritation potential of mancozeb technical has been investigated in an unpublished study. The study was performed to contemporary regulatory standards. Six New Zealand White rabbits (2 male, four female) each had 500 mg mancozeb technical (purity 88.2 % - other components not stated) applied to the skin under a semi-occlusive dressing for 4 h. No signs of skin irritation were observed (Unpublished, 1986l).

3.3.2.1.2 Eye

3.3.2.1.2.1 Zineb

A brief summary report, limited in detail, is available of an eye irritation study using 'technical' zineb. Undiluted 'technical' Zineb (100 mg - purity and composition not stated) was instilled in to the right conjunctival sac of 6 New Zealand White rabbits. The left eye of each animal acting as a control. The eyes were examined for irritation reactions on the cornea, iris and conjunctiva, 1,2,3,4 and 24 h post-instillation and daily for 14 d for signs of irritation. No signs of conjunctival irritation were observed. No information regarding corneal or iris irritation was reported (Unpublished, 1976h).

An unpublished summary report is available of an eye irritation study using an 80 % w/w zineb formulation. The report is brief and limited in detail. The zineb formulation (100 mg - composition not stated) was applied to one eye of each of six New Zealand White rabbits. No signs of eye irritation were observed (Unpublished, 1976g).

The eye irritation potential of zineb was examined in rabbits in the study by Blackwell-Smith *et al* (1953). An unstated amount of technical zineb was placed into one eye of each of 10 rabbits (breed not stated). Zineb was said to have 'produced mild irritation' in the tested eyes of all of the animals as evidenced by erythema (severity or grade of response not stated) within 2 h after treatment. It was stated that no oedema was observed and that the erythema had resolved within 6-8 h after treatment. It is possible that the transient mild effects seen were due to the mechanical properties of the applied material being a powder rather than to any chemical irritant potential of zineb. As a follow-up, a second study was carried out using the same procedure but with the zineb applied as a 0.19 % suspension in water (this preparation stated as being used for agricultural spraying). No signs of eye irritation were observed.

3.3.2.1.2.2 Zineb formulation 'a'

The eye irritation potential of formulation 'a' containing 3.3 % zineb was also investigated. Six (3 male and 3 female) New Zealand White rabbits each had 0.1 ml of the formulation instilled into one eye, and were subsequently examined at up to 7 d after treatment. The formulation produced very severe conjunctival reactions (up to grade 4 erythema and chemosis) such that 4 animals were killed for humane reasons at 24 h. In the two remaining animals, grade 1 chemosis persisted up to 7 d. Clearly formulation 'a' produced severe eye irritation (Unpublished, 1984a).

3.3.2.1.2.3 Mancozeb

The eye irritation potential of mancozeb technical has been investigated in an unpublished study. The study was performed to contemporary regulatory standards and GLP. Six New Zealand White rabbits (1 male, 5 female) each had 66 mg (amount in 0.1 ml) mancozeb technical (purity 88.2 % - other components not stated) applied to one eye. No effects on the cornea or iris were observed. Conjunctival redness, chemosis and discharge (all generally of grade 2 severity) were observed in all animals within 1 h post treatment. These were still present at the same or slightly less severe levels, at 24-48 h in all rabbits but had resolved in all but two at day 3 after treatment. Grade 1 redness in two rabbits and grade 2 discharge in one persisted to 7 d but had resolved by 14 d after treatment. Overall, in this study, mancozeb technical induced moderate eye irritation in rabbits (Unpublished, 1986m).

3.3.2.1.3 Respiratory Irritation

3.3.2.1.3.1 Zineb

No data were submitted.

3.3.2.1.3.2 Mancozeb

No data were submitted. In a single dose inhalation study, marked mucosal necrosis of the larynx was seen on histopathological examination of animals that died following inhalation exposure to mancozeb.

3.3.2.2 STUDIES IN HUMANS

The skin irritation potential of zineb in human volunteers was examined in an older study described in a published report (Blackwell-Smith *et al*, 1953). The material tested was described as a '65 % commercial wettable powder', although no further information was provided on the composition or purity,. This was applied on to the inner surface of the forearms of 50 volunteers on a ¼ inch cotton square, which had been dipped into water. This was then covered with a 1-inch (6.45 cm^2) square of aluminium foil and held in place for 48 h with adhesive tape. No signs of skin irritation were observed in 49 individuals. One person had small papular eruptions (which were aggravated by bathing) at the application site that persisted for one week.

3.3.2.3 SUMMARY OF IRRITATION

There are few reports available on the skin irritation potential of zineb. Those that are available are old and have limitations in the reporting. From the few data available (two animal and one human study) the indications are that zineb is unlikely to possess any significant skin irritation potential. This view is supported by the data for the structurally related mancozeb. The data for eye irritation potential are equally as poor but suggest little eye irritation potential for zineb. In contrast a study conducted to modern standards with good reporting indicates that mancozeb has moderate eye irritation potential, raising questions about the adequacy of the eye irritation data available for zineb. No data were available on the potential for zineb to act as a respiratory tract irritant. Exposure concentrations of mancozeb producing lethal effects were found to induce histopathological damage to the larynx. However, it is unclear if such effects occurred at lower non-lethal exposure concentrations. Overall, no conclusions on the respiratory tract irritation potential of zineb can be drawn.

A formulation of zineb (formulation 'a') demonstrated a low potential to cause skin irritation when applied to the skin of rabbits. Formulation 'a' caused severe eye irritation (up to grade 4 redness and chemosis) in a study in rabbits. No data on respiratory tract irritation were available for this formulation.

3.3.3 SENSITISATION

3.3.3.1 SKIN

3.3.3.1.1 Studies In Animals

3.3.3.1.1.1 Zineb

A single published report was found in which the skin sensitisation potential of zineb was investigated in Hartley guinea pigs using the Magnusson and Kligman maximisation test (Matsushita *et al*, 1976). The report generally lacks detail, particularly regarding methodology. Zineb, as a technical grade commercial form, was used in the study, diluted or emulsified with distilled water. Groups of 10 guinea pigs were used and the procedures employed were stated to be the same as those used in the original publication by Magnusson and Kligman. No mention is made of control groups in the report; it is likely that none were included. Concentrations of 5 % and 25 % zineb were used for intradermal and topical induction respectively, the latter on the basis of a preliminary sighting study which indicated that 5 % was a threshold for skin irritation in the guinea pigs. Challenge concentrations of 0.5 % and 2 % were used. Response data (tabulated) indicated that all the animals showed a response on challenge at 2 % and 9/10 at 0.5 %. These data indicate that zineb has skin sensitizing potential. However, the data should be interpreted with some caution since apparently no negative control group was employed. Thus it is unclear whether or not the system employed was reliable, although only weak reactions were recorded for some substances such as ETU and the sodium salt of dimethyldithiocarbamic acid. Cross-reaction studies, carried out as part of the same investigation showed that all guinea pigs induced with zineb also responded to challenge with mancozeb and also the converse relationship was also demonstrated (see below).

3.3.3.1.1.2 Zineb formulation ‘a’

The skin sensitisation potential of formulation ‘a’ containing 3.3 % zineb has been investigated in a poorly conducted Buehler study. Groups of 20 Dunkin-Hartley guinea pigs were used. Induction was undertaken initially using the undiluted formulation (based on the data from a preliminary sighting study) but after two applications the material was applied as a 50 % (w/w) concentration in acetone due to irritant effects (no data presented for these). Controls were treated with acetone alone. The initial challenge concentration was also 50 % (w/w) in acetone. This produced marked responses in both test and control groups (acetone alone did not induce any response) in equal numbers of animals. Re-challenge at 25 % and 10 % also resulted in responses of equally severity and incidence in both groups. Overall, no conclusions regarding the skin sensitisation properties of zineb (or the formulation) can be reached from this study (Unpublished, 1984a).

3.3.3.1.1.3 Mancozeb

The skin sensitisation potential of mancozeb has been investigated in a modified Buehler test using 10 treated and 10 control Dunkin-Hartley guinea pigs. The study was performed to contemporary regulatory standards and GLP with mancozeb technical (purity 88.2 % - other components not stated). A 50 % w/w solution of mancozeb was used for both induction and challenge treatments based on the outcome of a preliminary sighting study. Overall 9 induction treatments were performed, with the treatment site being relocated for the sixth to ninth treatments due to severe skin reactions (including necrotic patches) at the initial induction site. Challenge with mancozeb produced grade 1-2 erythema and grade 1 oedema in seven treated animals but no reactions in any of the control animals. A clear positive response was obtained indicating mancozeb to be a skin sensitiser under the conditions of the test (Unpublished, 1986n).

Mancozeb was also investigated in the published study described above for zineb (Matsushita *et al*, 1976) using the same methodology, including the induction and challenge concentrations. All animals responded to challenge with mancozeb at both concentrations (0.5 % and 2 %).

3.3.3.1.2 Studies In Humans

A number of published reports are available on human experience of skin sensitisation with zineb and mancozeb (Bartalini, 1962; Burry, 1976; Iliev and Elsner, 1997; Jung *et al*, 1989; Koch, 1996; Lisi and Caraffini, 1985; Lisi *et al*, 1987). Most of these are case reports that give very brief details of individuals who have presented with contact dermatitis, usually following a period of occupational exposure to mixtures of active ingredients in one or more products.

Jung *et al*, 1989 reported on a series of 19 cases of allergic dermatitis, believed to be caused by pesticides during 1985-1986 in the former German Democratic Republic. Although some data were provided on each of these individuals including some work history, no information was given on their atopic status or any previous history of allergic episodes or skin disease prior to the development of their current dermatitis. Patch testing, few details of which were provided in the report, was carried out in each case. In eight cases positive responses were obtained with zineb and/or mancozeb. It is unclear whether or not other active ingredients had also been tested in these individuals and thus specificity of response to zineb/mancozeb is unclear. Of the eight cases, two were in male chemical workers who had been involved with the production of zineb and maneb for 1-2 months prior to developing dermatitis. The remaining cases were all in females who worked either as florists or gardeners. In these cases it was believed that contact had occurred through the use of the pesticide formulations on flowers, though in some cases these individuals also responded when challenged to a number of other formulations and plant extracts. In general, given the limited information in the reports, it is difficult to judge whether the positive response to patch testing was due to primary irritation, sensitisation to the substance or a cross-reaction following sensitisation to another, possibly structurally related material. It is unlikely that skin irritation was involved, as the potential for this appears to be low (see Section 3.3.2). Cross-reaction is possible as in some cases people were found to react with a number of substances. The most compelling cases are those of the two production workers since they were apparently exposed for a relatively short time (1-2 months), and were known to work specifically with zineb and maneb.

Lisi *et al*, 1987, describe the results of a study in which they undertook patch testing in 652 patients with skin disorders to investigate the irritant and skin sensitisation potential of pesticides used in Italy. Included in the study population were current agricultural workers (180), ex-agricultural workers (43) and 429 'others'. Of the 652 people, 274 had presented with contact dermatitis (mainly of the hands) with the rest of the patients being admitted to clinics with non-allergic skin disorders. Patch testing, conducted with a large range of pesticides including zineb and mancozeb (both at 1 % in petrolatum), was used to determine irritant and sensitisation reactions. Zineb was tested in a total of 389 people (125 current agricultural workers; 39 ex-agricultural workers; 225 'others'). One person from each of the current agricultural workers and 'others' groups were recorded as showing an allergic reaction; no irritant responses were recorded. Mancozeb was tested in a total of 149 people and no irritant or allergic responses were recorded.

Burry (1976), Iliev and Elsner (1997), Koch (1996) and Lisi and Caraffini (1985) all provide case reports of individuals. The reports vary from very brief to more detailed but all with essentially similar characteristics. In each case the individual presented with contact dermatitis following exposure to pesticide mixtures at work. In all cases the mixtures

contained mancozeb as an active ingredient and the individuals were patched tested with a range of substances including mancozeb and zineb. In all but one case (Lisi and Caraffini, 1985), patch testing with zineb was found to produce a positive response, indicating that the individual cross-reacted on challenge (probably following exposure to the mancozeb). Other active ingredients and products, when tested, were also generally found to induce positive responses. Although positive results were obtained on challenge with zineb in these cases, it is difficult to draw any conclusions regarding its skin sensitisation potential directly as in no case was the individual primarily exposed to the active ingredient.

One older report describes in limited detail cases of dermatitis possibly associated with zineb (Bartalini, 1962). Two cases are briefly described of individuals who worked in the manufacture and packaging of zineb in Italy. In each case the individual developed dermatitis within either 2 d or about one month after beginning work with zineb. The skin conditions resolved with removal from the work and treatment in the case of the individual exposed for the shorter time but persisted, despite treatment, in the other person. Both individuals were patch tested. Mild effects were apparently seen in the worker exposed for the shorter time but an intense response was seen in the other individual. It was stated that other workers were also patch tested but none was found to respond. Given the short time interval in the first case, it is difficult to believe that this was due to skin sensitisation. The report also presents, in a general discursive manner with little supportive detail, information on the problems encountered in the fungicide spraying campaign in Italy during 1956-57 when zineb was apparently first introduced. Cases of dermatitis were recorded during this time that were attributed to intense (both in terms of duration and dose) exposure to zineb in viticulture, with the Author estimating that around 2 % of exposed workers developed dermatitis; these included what were cited as 'very minor cases'. Some quantitative data were presented relating to the patch testing of workers with or without dermatitis. Of 72 with dermatitis tested (selection criteria not specified), 31 showed no response, 24 a weak response and 17 an intense response. Of 30 without dermatitis (again, selection criteria not specified), 26 showed no response, 4 a weak response and none an intense response. The report states that when precautionary measures were taken during subsequent spraying campaigns then the number of cases of dermatitis was found to decline. The report also contains information, again very briefly presented, on experimental studies carried out in human volunteers. In general all these studies employed zineb up to 2 %, involved either topical application or intradermal injection over relatively short time periods (up to 10 d) and gave negative results. This report presents essentially anecdotal information on the potential for dermatitis in people exposed to zineb and as such great caution should be exercised in the drawing of any conclusions. However, it is interesting to note that the paper describes the initial use of zineb in a relatively uncontrolled manner with population exposed over extended periods of time with an outbreak of dermatitis apparently not previously seen in these workers.

No reports of human sensitisation to zineb were found in either the EPIDERM (Occupational Skin Surveillance Survey, 1993 to 1998) or OPRA (Occupational Physicians Reporting Activity, 1994 to 1998) databases.

3.3.3.2 RESPIRATORY SENSITISATION

No data available.

3.3.3.3 SUMMARY OF SENSITISATION

A number of human case reports are available in which individuals have presented with contact dermatitis and subsequently been found to respond to skin challenge with zineb. However, none of these cases provides conclusive evidence that zineb itself possess skin sensitisation potential. Zineb was found to produce positive results in a limited maximisation test in guinea pigs. The structurally related mancozeb also produced positive results in the same guinea pig study and was also shown to be a skin sensitiser in a more recent Buehler study. Overall, although the database is somewhat limited, taken altogether the available information indicates that on balance zineb is likely to possess skin sensitizing potential. No data are available on whether or not zineb is likely to be a respiratory sensitiser.

Formulation 'a' containing 3.3 % zineb has been tested in a poor Buehler study. No information regarding the sensitisation potential of this formulation can be drawn from this study.

No reports of human sensitisation to zineb were found in either the EPIDERM (Occupational Skin Surveillance Survey, 1993 to December 1998) or OPRA (Occupational Physicians Reporting Activity, 1994 to 1998) databases.

3.3.4 REPEAT DOSE STUDIES

3.3.4.1 SUB-CHRONIC STUDIES (< 90 D DURATION)

3.3.4.1.1 Oral

3.3.4.1.1.1 Zineb

3.3.4.1.1.1.1 Rats

A study in rats comparing the effects of low doses of zineb and ethylenethiourea (ETU) on the thyroid is available (Nebbia and Fink-Gremmels, 1996). Groups of 10 male Wistar rats were fed zineb or ETU in the diet at concentrations of 0, 50, and 500 ppb for 5 d. Using accepted guidelines (Unpublished, 1990d), these dietary concentrations are estimated to be equivalent to dosages of 0, 0.05 and 0.005 mg kg d^{-1}. The zineb was reported without further description to be 92 % pure and the ETU 98 % pure. On day 6, following overnight fasting, rats were weighed and blood samples taken. Blood serum was analysed for thyroxine (T_4), triiodothyronine (T_3), free thyroxine (FT_4), thyroid stimulating hormone (TSH), glucose (blood level) and cholesterol. Zineb showed no effect on levels of T_3, a slight increase in TSH and slight decreases in T_4 and FT_4 that were neither dose-related nor generally statistically significant. Similar results were observed with rats exposed to ETU with 2 exceptions: a slight statistically significant decrease in the level of FT_4 that was not dose-related; and glucose levels were comparable to controls. Overall, only slight changes in

parameters of thyroid function were observed and in all cases showed no dose-response relationship. Overall, these findings are of doubtful toxicological significance.

Brief details of a study performed in rats receiving zineb in the diet are available (Vos *et al*, 1982). Groups of 6 weanling (body weight 40-60 g) male Wistar-derived rats received 650, 2500 or 10000 ppm zineb in the diet for a period of 3 weeks. Using accepted default food consumption and body weight default values (Unpublished, 1990d), these dietary concentrations are estimated to be equivalent to 195, 750 and 3000 mg kg^{-1} d^{-1}, respectively. The zineb used was 'technical' grade and of 90 % purity. Body weights and food intake were recorded routinely. At 3 weeks the animals were killed and liver, kidney, pituitary, adrenals, thyroid, testes, thymus, spleen, mesenteric and popliteal nodes weighed and fixed. In addition, total and differential leukocyte counts, and serum IgM and IgG levels determined. Details on the frequency and severity of the findings were not given in the tabulated results Section. It appears that in the 2500 ppm group (and presumably in the 10000 ppm group), increased relative thyroid weight with histopathological change, decreased serum T_4 level, and decreased relative thyroid weight were observed. No findings were observed at a dietary concentration of 600 ppm (dose estimated to be 195 mg kg^{-1} d^{-1}).

A limited study looking at effects on the thyroid has been published (Blackwell-Smith *et al*, 1953). The zineb used was described as a '65 % commercial wettable powder', although no further information was provided on the composition or purity. Groups of 20 young 'albino' male and female rats (strain not specified) were fed zineb in the diet at concentrations of 0, 500, 1000, 2500, 5000 and 10000 ppm for 30 d. Using accepted guidelines for default values (Unpublished, 1990d), these dietary concentrations are estimated to be equivalent to dosages of 0, 50, 100, 250, and 1000 mg kg d^{-1}. At 10, 20 and 30 d, 5 animals/sex/concentration were killed and thyroid glands removed and weighed. Histopathological examination of the thyroid was performed to ascertain and rank the degree of epithelial hyperplasia, follicular enlargement and loss of colloid. Body-relative thyroid weights (not specified) were statistically significantly increased ($P<0.05$) in males at $\geq$ 5000 ppm at 10 d, $\geq$ 1000ppm at 20 d and $\geq$ 500 ppm at 30 d and in females at 10000 ppm at 10 d, $\geq$ 500 ppm at 20 d and 500 ppm and 10000 ppm at 30 d. In females, greater statistical significance (not further defined) was achieved only at 10000 ppm at each time interval. Thyroid epithelial hyperplasia was noted in 2/5 males and 1/5 females fed 10000 ppm zineb for 30 d.

Groups of 4 male Wistar rats were dosed by oral gavage daily with 0 or 1000 mg kg^{-1} d^{-1} zineb (purity not reported) in peanut oil for 30 d in a limited study (Raizada *et al*, 1979). At the end of this period, only the thyroid, pituitary and testis were weighed and examined histopathologically. No deaths occurred and no clinical signs of toxicity were observed; body weight data were not reported. Compared to controls there was an increase in pituitary weights (relative weight increased 34 %; absolute weight up 35 %). The results of histopathological examination of the pituitary were not reported. In the thyroid, a large increase in weight (relative weight increased by 125 %; absolute weight up by 127 %) was accompanied by the finding of 'marked' follicular hypertrophy. Testis weights were comparable to controls but microscopic examination showed that 'most' seminiferous tubules were necrotic although the Leydig cells appeared to be unaffected.

The effect of zineb treatment on hepatic xenobiotic metabolism has been studied following oral dosing (Siddiqui *et al*, 1991). Groups of 5 albino rats (strain not specified) received a daily oral gavage dose of 250 mg kg^{-1} zineb for 4 weeks. The zineb (apparently a commercial preparation) was stated to be 82 % pure (content of the other 18 % was not

given) and was administered as a suspension in 1 % gum acacia; controls received gum acacia alone. The rats were killed at 18-20 h after last treatment and the effects on hepatic mixed function oxidase (MFO) evaluated by assessing the activity of aminopyrine N-demethylase (APDM), p-nitroanisole O-dealkylase, aniline hydroxylase (AH) and benzo(a)pyrene hydroxylase. No changes were seen in any parameters of enzyme activity. This result differs from the observation by the same Authors of liver xenobiotic metabolism inhibition 1 or 4 h after administration of a single dose of 100 or 250 mg kg^{-1} (see Section 3.3.1.1.1.1).

A limited study has been published which focused exclusively on indicators of enzyme activity in the liver produced by repeated oral exposure to zineb in rats (Lowy *et al*, 1977). Groups of 8 male Wistar rats received dietary levels of 0 (16 animals), 6, 15, 60, 600 and 3600 ppm zineb (purity 92 %, composition not further described) for 28 d. Using an estimate mean body weight based on the data provided in the report and an estimate of daily food consumption of 15 g d^{-1} (Unpublished, 1990d), these dietary concentrations are equivalent to 0.5, 1.2, 4.7, 47 and 279 mg kg d^{-1}, respectively. At the end of the dosing period and following overnight fast, livers were excised, weighed, homogenised and enzyme activities of APDM and AH and microsomal concentrations of cytochromes P450 and b_5 were determined. Details of liver weight measurements related to dietary concentration were not presented in the paper. However, the Authors reported that only small, non-statistically significant increases in relative liver weight of up to 4 % were observed in exposed animals (dietary levels not stated) compared to controls. Enzyme concentration or activity was measured against 3 or 4 parameters of liver function: 100 mg microsomal protein; 1 g liver; whole liver weight; and where appropriate, cytochrome P450 concentration in the case of enzyme activity. No effects were seen at doses of up to 60 ppm, equivalent to ~5 mg kg^{-1} d^{-1}. Decreases in both APDM and AH activities showed similar dose-response relationships showing statistical significance ($p<0.05$) for all parameters at 600 ppm (APDM -18 to -26 %; AH -24 to -32 %) and at 3600 ppm (APDM -40 to -56 %; AH -35 to -53 %). Levels of cytochrome b_5 concentration were comparable to controls but dose-related decreases in cytochrome P450 concentration were statistically significant ($p<0.05$) for 1 parameter at 600 ppm (9.7 %) and all 3 parameters at 3600 ppm (range 23 - 26 %; $p<0.01$).

Indicators of hepatic liver enzyme activity were measured in a study of the effects of diet on zineb metabolism in the rat (Pelissier *et al*, 1981). Groups of Wistar rats (number unspecified) were fed energy balanced feed containing 18 % or 9 % protein (casein) for 2 weeks. Following this period, each group of rats (18 % or 9 % casein) was then subdivided into a control group and a treated group fed dietary zineb (92 % pure) at a level of 600 ppm for 4 weeks. Following termination, liver MFO was assayed by measuring the activities of the quantities of P-450 and cytochrome b_5 and the activities of, APDM, AH and NADPH-cytochrome c reductase. Compared to the relevant control group, zineb treatment lowered the activities of APDM and AH calculated per unit of microsomal protein, but not when calculated on the basis of P-450 units. Zineb treated groups showed no statistically significant changes in the quantities of P-450 and cytochrome b_5 and no change in the activity of NADPH-cytochrome c reductase.

Groups of 6-18 male and female Wistar rats received approximately 0, or 5000 mg kg^{-1} d^{-1} 'Cynkotox' (approximately 71 % zineb, other constituents unknown) by oral gavage daily for 10 d (Godlewski, 1981). On completion of the exposure period, the brain was removed for detailed histopathological examination and analysis of a range of enzyme activities. There were no investigations of other tissues and no indication was given regarding other signs of

systemic toxicity, although given that the oral LD_{50} for single exposure to zineb is around 8000 mg kg^{-1}, the exposure levels used were clearly high.

Changes in treated animals included proliferation of glial cells (particularly oligodendrocytes) noted throughout the brain, and hypertrophy of astrocytes. Pyknotic nuclei were also observed in oligodendrocytes. The magnitude of changes in enzyme activities was not presented, but the main changes were stated to include an increased activity of non-specific esterase in glial cells and astrocytes, but not of acetylcholinesterase.

Overall, this study demonstrates the observation of lesions in the brain with some associated changes in enzyme activity. Due to the limited observations, it is difficult to determine whether or not the changes in enzyme activity were as a secondary consequence of other systemic effects or were directly related to the brain lesions. As seen from the use of one, high dose level, the full dose-response relationship for these effects has not been explored.

A similar profile of widespread brain lesions and changes in enzyme activities was seen in an earlier study conducted to an identical protocol (Maziarz, 1981).

Electron microscopy was used to study neutrophil morphology in the bone marrow of rats exposed to zineb (Tochman *et al*, 1981). Groups of 5 male white rats (strain unspecified) were dosed by oral gavage with an aqueous suspension of 30 mg kg^{-1} zineb 6 d $week^{-1}$ for 1, 2 and 3 months respectively. The dose used was in terms of content of the active ingredient, zineb, present at 71 % in a preparation called 'cynkotox'; the remaining 29 % of the preparation was unspecified. At the end of the dosing period and following a 1 day fast, bone marrow was extracted from the femur and fixed for examination by electron microscopy. Some slides were also treated with diaminobenzidine (DAB) to show myeloperoxidase activity and to distinguish between first- and second-order granules. Concurrent control animals were not used and the Authors compared their findings with control animals used in previous experiments. Findings included disruption (thinning and missing portions) to neutrophil cell membranes, and in addition, there were incidences of fragmented membranes of intracellular organelles such as ribosomes and endoplasmic reticulum and swollen mitochondria. In the 1 month exposure group, neutrophil promyelocytes showed a decrease in the presence of DAB-reaction products in the endoplasmic reticulum membranes, Golgi apparatus and first order granules and upon examination of the 2 other test groups, that the amount or DAB reaction products present in first-order granules was decreased in relation to the duration of exposure. In the absence of reliable control data and more thorough reporting of the data, the quality of this study cannot be ascertained, and moreover, the toxicological significance of these findings is unclear.

3.3.4.1.1.1.2 Rabbits

Nebbia *et al* (1995) studied the effect of zineb treatment following dietary administration. Groups of 8 New Zealand White rabbits received diet containing 0, 3000 or 6000 ppm zineb for 90 d. Using estimated default values (Unpublished, 1990d), these dietary concentrations are equivalent to 0, 90 or 180 mg kg d^{-1}. The 'technical grade' zineb was stated to be 92 % pure but without further description of the identity. Clinical behaviour, food consumption and weight gain were routinely monitored. In addition, the following examinations were performed: haematologic and serum biochemical analysis; gross necropsy; liver, thyroid and testes weights; histopathological examination of a wide range of tissues.

Decreased body weight gain was observed in treated animals (approximately 15 % and 20 % at 3000 and 6000 ppm zineb, respectively) with concurrent decreases in food consumption (25 and 59 % at 3000 and 6000 ppm zineb, respectively). In the animals of the 6000 ppm treatment group only, statistically significant decreases (>30 %) were recorded for haematocrit, haemoglobin concentration, erythrocyte and total white blood cell counts, and statistically significant increases in total lipids (214 %) and serum cholesterol (315 %). TSH levels showed no statistical differences between groups; however, T_3 levels showed dose-related statistically significant ($P<0.05$) decreases (0.41, 0.25, and 0.21 $ng.ml^{-1}$ at 0,3000 and 6000 ppm zineb, respectively) and T_4 levels were significantly decreased (by > 60 %) in the 6000 ppm treatment group. Using whole liver samples, statistically significant decreases in the levels of lipid (29 % decrease in 6000 ppm treatment group) and triglyceride (50 % and 72 % decreases at 3000 and 6000 ppm zineb, respectively) were observed. Using calorimetry, no effects on the phospholipid content of liver microsomal fraction were observed. In samples of homogenised testis, a dose-related increase in GST activity was seen (24 % in 6000 ppm treatment group vs. controls) but levels of lactate dehydrogenase (LDH) and LDH-X were comparable between all groups. The thyroid showed statistically significant dose-related increases in absolute weight, (increased 2.4 fold and 8-fold at 3000 and 6000 ppm zineb, respectively) with similar increases in relative weight. In animals fed with 6000 ppm zineb, a slight increase in liver relative weights (27 %) and a slight decrease in testes absolute weight (23 %) may reflect the malnutrition seen in these animals. Histopathological findings were poorly reported. In the thyroid, incidences of colloidal struma (goitre), occasionally cystic, and flattening of the follicular epithelium were observed in animals of the 6000 ppm group and similar although less severe findings were apparent in the 3000 ppm group. In the liver slight glycogenesis was observed whilst in the spleen hemosiderosis was observed in the 6000 ppm group animals.

The same team of workers studied the effect of zineb treatment on hepatic xenobiotic metabolism following dietary administration to rabbits (Nebbia *et al*, 1993). Again, groups of 8 New Zealand White rabbits received a diet containing 0, 3000 and 6000 ppm zineb for 90 d. Using estimated default values (Unpublished, 1990d), these dietary concentrations are equivalent to 0, 90 or 180 mg $kg\ d^{-1}$. The zineb, of technical grade, was stated to be 92 % pure with <0.25 % ETU present. At the end of the feeding period, the animals were killed following an overnight fast, the liver removed and weighed and measurements made of a range of hepatic parameters including the activities of xenobiotic metabolising enzymes (MFOs, glutathione transferase and reductases). Absolute liver weight was unaffected, however, relative liver weight was slightly increased (26 %; $P\leq0.05$) in the 6000 ppm group (body weight data not reported). Zineb did not affect cytochrome P450, total haem, microsomal or protein content. There was a slight increase (24 %; $P<0.05$) in cytochrome b_5 content in animals of the 6000 ppm group. Dose-related decreases in MFO activities became statistically significant at the higher test concentration with decreases in activities of NADPH cytochrome c reductase (about 50 %), aminopyrine N-demethylase (about 40 %), aniline-4-hydroxylase (about 60 %), and ethoxycoumarin O-deethylase (about 75 %), whilst membrane-bound microsomal carboxyesterase indophenyl acetate esterase was unaffected. Dose-related changes in hepatic glutathione content (statistically increased) and glutathione S-transferase (statistically decreased) were observed, whilst selenium-independent glutathione peroxidase was slightly decreased (statistically significant in 6000 ppm treatment group only). Selenium dependent glutathione peroxidase was only slightly increased.

Parameters of hepatic enzyme activities were further investigated in animals of the 6000 ppm treatment group. A decrease in activity of 38 %($P<0.001$) was determined for μ class GSH-

transferase whilst π class GSH-transferase activity was unaffected. Thiol transferase activity (regulates GSH intracellular redox reactions) was increased by 55 % ($P<0.001$) whilst GSH-reductase was unaffected. Glyoxylase I activity was decreased (31 %; ($P<0.01$), however, glyoxylase II activity was unaffected. In this study, repeated exposure to zineb showed inhibition of specific MFOs, effects on specific enzymes controlling glutathione levels not seen in acute studies and no effects on microsomal-membrane bound carboxylesterase.

3.3.4.1.1.2 Mancozeb

3.3.4.1.1.2.1 Rats

In a standard, well-conducted study, to GLP standards, groups of Sprague-Dawley rats (20 animals per sex in control and top dose groups and 10 animals per sex in interim dose groups) were continuously administered technical mancozeb (88 % purity, remaining composition not stated) in the diet at concentrations of 0, 25, 100 or 400 ppm for up to 13 weeks. The mean calculated doses of mancozeb received were 0, 2, 7 or 29 mg kg^{-1} d^{-1} in males and 0, 2, 8 or 33 mg kg^{-1} d^{-1} in females (Unpublished, 1989a).

All animals were monitored throughout for clinical signs of toxicity, body weight and food consumption. Ten animals per sex from each group were killed after 13 weeks of dosing. The remaining animals were killed after a recovery period of 4 weeks. Extensive haematological and clinical chemistry (including T_4 and T_3) analyses were conducted at week 13 and recovery week 4. At each sacrifice time, gross pathological and microscopic examinations were conducted on all tissues from all animals. In addition, in week 13 pre-sacrifice ophthalmoscopy was conducted on all animals.

All animals survived to study termination. No clinical signs of toxicity were observed in any of the animals. No significant changes in body weights or food consumption were observed in any animals up to 100 ppm or in males at 400 ppm, compared with controls. Over the treatment period females at 400 ppm had statistically significantly reduced mean body weight (15 %) compared with controls, but food consumption was unaffected. Following 4 week recovery females at 400 ppm displayed a significant increase in body weight compared with controls. No treatment-related ocular effects were observed in animals at any exposure level. No changes in haematology, clinical chemistry or urinalysis occurred at either sacrifice time across the exposure groups, except for a marginal, but statistically significant, reduction in neutrophil numbers in females at 100 (37 %) and 400 ppm (40 %) at week 13.

No changes in thyroid function analysis were observed in animals receiving 25 ppm at both examination times. At week 13, T_4 levels were statistically significantly reduced by 19 % in males at 400 ppm. A reduction in T_4 levels, but not statistically significant was also observed in females at 100 and 400 ppm (both 15 %). At week 4 of recovery T_4 levels in all animals were comparable with controls. At week 13 a slight, but statistically significant increase in T_3 levels (22 %) was detected in females at 400 ppm. At week 4 of recovery levels were comparable with controls. No changes in T_3 levels in other groups were reported. No changes in organs weights, macroscopic or histopathological findings were observed in animals across the exposure groups at either sacrifice time. Overall, slight reductions in plasma T_4 levels were observed in males and females at 400 ppm. An oral NOAEL of 100 ppm (equivalent to 7-8 mg kg^{-1} d^{-1}) can be identified from this study.

In another unpublished study, groups of Sprague-Dawley rats (14 per sex per group) were continuously administered mancozeb (84 % purity, remaining composition not stated) in the diet at concentrations of 0, 30, 60, 125, 250 or 1,000 ppm for up to 3 months. The mean calculated doses of mancozeb received were 0, 2, 4, 7, 15 or 57 mg kg^{-1} d^{-1} in males and 0, 2, 4, 9, 18 or 75 mg kg^{-1} d^{-1} in females. All animals were monitored throughout for clinical signs of toxicity, body weight and food consumption. At study termination blood from 10 animals per sex per group was taken for haematological, clinical chemistry and thyroid function analysis. The animals were killed, all standard organs were weighed, macroscopic and histopathological investigations conducted on a wide range of tissue and organs. Liver samples from 6 animals per sex per group were used for analysis of mixed function oxidase (MFO) activity by both aniline hydroxylation and AP N-demethylation (Unpublished, 1986e).

All animals survived to study termination. No clinical signs of toxicity were observed in any of the animals. No changes in body weights or food consumption were observed in animals up to 250 ppm, compared with controls. Decreased mean body weights of less than 15 %, and reduced food consumption were noted in both sexes at 1,000 ppm. No treatment-related ocular effects were observed in animals at any exposure level. No changes in organ weight were observed in animals up to and including 250 ppm, compared with controls. Relative liver and thyroid weights were statistically significantly increased (10-23 % and 33-60 %, respectively) in both sexes at 1,000 ppm. Absolute and relative spleen weights were increased in females at 1,000 ppm, by 15 % and 22 %, respectively. No changes in haematology, clinical chemistry or urine analysis occurred at any exposure levels. No changes in thyroid function analysis were observed in animals up to and including 125 ppm. Serum thyroxine (T_4) levels were statistically significantly reduced by 28 % in females at 250 ppm and in males (34 %) and females (43 %) at 1,000 ppm**.** TSH levels were increased (but not statistically significantly) 94 % in females at 250 ppm and in males (261 %) and in females (169 %) at 1,000 ppm**.** No treatment-related effects on serum triiodothyronine (T3) levels were observed.

No change in MFO activity was determined in animals up to and including 250 ppm. MFO activity was decreased, but not statistically significantly, when measured by aniline hydroxylation by 31 to 35 % in males and 34 to 40 % in females at 1,000 ppm. AP N-demethylation activity was not affected. No effect on hepatic microsomal protein concentration was observed in animals across the exposure range.

Histopathological investigation showed no evidence of treatment-related effects in any organs of animals up to 250 ppm. At 1000 ppm, treatment-related effects in the thyroid, pituitary glands and liver were reported as; thyroid follicular cell hyperplasia (slight to moderate) in both sexes; an increased severity of hypertrophied cells (generally marked to moderate) in the anterior pituitary in males, and centrolobular hypertrophy of minimal severity in 2 males.

Overall, the results of this study indicate that the thyroid and the liver are the key organs affected by mancozeb; the effects observed include marked increases in liver weight associated with microscopic cytoplasmic changes, together with disturbances in MFO activity. Slight thyroid hyperplasia and reductions in plasma T_4 levels are also observed; these effects are considered to be a direct effect of mancozeb and/or its metabolites on the thyroid. An oral NOAEL based on reductions in T_4 levels in females at 125 ppm (equivalent to 7 mg kg^{-1} d^{-1}) can be identified from this study.

In an unpublished study conforming with GLP, groups of 10 male and 10 female Crl:CD rats received 0, 20, 125, or 750 ppm mancozeb (79 % pure; other components not stated) by dietary administration for 90 d. In addition, one group of 10 males received 5000 ppm for 90 d, and a group of 10 females also received 5000 ppm for 2 weeks, but due to excessive mortality received untreated diet for the remaining duration of the study. A further 16 females were re-introduced 2 weeks before the end of the study and also received 5000 ppm to provide comparative data with other animals killed at the same point in time. Particular attention was paid to histological investigation of nervous tissue (brain, spinal cord, proximal sciatic nerve, tibial nerve, cervical and lumbar dorsal root ganglia, sural nerve); there were no other macroscopic or microscopic investigations (Unpublished, 1991a).

The mean daily intake correlated to approximately 0, 1.4, 8, 50, and 340 mg kg^{-1} d^{-1} over the 90-day treatment period for males and 0, 1.7, 11, and 64 mg kg^{-1} d^{-1} for females receiving up to 750 ppm. The first two weeks of treatment for females at 5000 ppm equated to about 480 mg kg^{-1} d^{-1} and the last two weeks to around 410 mg kg^{-1} d^{-1}.

One male and 4/10 females at 5000 ppm (340/480 mg kg^{-1} d^{-1}) died within the first 4 weeks. At this exposure level clinical signs of toxicity amongst males included loss of use of hind limbs and a noticeable loss of muscle in the rear legs during the first three weeks, although by day 60 this was no longer apparent. For females at 5000 ppm, the loss of use of hind limbs and muscle wasting was of sufficient severity to warrant the cessation of mancozeb exposure after 2 weeks. Use of hind limbs was regained within about a week of receiving untreated diet. No treatment-related clinical signs of toxicity were observed in other groups.

In males at 5000 ppm (340 mg kg^{-1} d^{-1}) there was a marked, statistically significant reduction in body weight gain compared with controls over the 90-day period (63 % lower). Group mean body weight loss was seen in the females that received 5000 ppm (480/410 mg kg^{-1} d^{-1}) for two weeks. At 750 ppm (64 mg kg^{-1} d^{-1}), weight gain amongst females was reduced, but did not attain statistical significance (16 % lower). Weight gain of other groups was not affected. Similarly, food consumption was reduced only in males at 5000 ppm (43 % lower than controls) and in females at 5000 ppm (for the two weeks that they received it, by around 30 %). The effects on body weight gain and food consumption may be related to the loss of use of hind limbs.

Histological examination showed that the tibial, sciatic, and sural nerves were particularly affected. Males at 5000 ppm (340 mg kg^{-1} d^{-1}) showed myelin ‘bubbles’ (swollen vacuolated sheathes around a shrunken axon), demyelination, Schwann cell proliferation, and/or neurofibrillary degeneration in the sciatic nerve of 4/9, tibial nerve of 6/9, and sural nerve in 8/9 animals. In addition, muscle atrophy was observed in 7/9 males at this exposure level. At 750 ppm (50 mg kg^{-1} d^{-1}), similar histopathologically observable nerve lesions were also seen in the tibial nerve of 8/10 animals, and the sural nerve of 10/10. The sural nerve was also affected in 1/9 males at 125 ppm (8 mg kg^{-1} d^{-1}), but was not affected in male controls; however, the incidental observation in males at 125 ppm is considered of doubtful importance given incidental occurrence in female controls (see below).

Tibial nerve lesions (myelin bubbles etc) were seen in 2/10 females at 750 ppm (64 mg kg^{-1} d^{-1}), 1/6 females at 5000 ppm (480 mg kg^{-1} d^{-1}), and 1/10 controls. Sural nerve lesions were seen in 7/10 animals at 750 ppm, 6/6 at 5000 ppm, and 1/10 controls. In contrast to the males, there were no lesions seen in the sciatic nerve and no animals at

125 ppm (11 mg kg^{-1} d^{-1}) were affected. Despite the evident problems with hind limbs of females in this study, muscle atrophy was reported in only 1/6 at 5000 ppm.

Overall, mortality, loss of use of hind limbs, muscle atrophy, and reduced body weight gain were observed amongst males and females at 5000 ppm (about 340 or 410-480 mg kg^{-1} d^{-1} respectively). Histopathologically, nerve lesions were seen at 750 (50 to 64 mg kg^{-1} d^{-1} and 5000 ppm. Overall, no adverse nervous tissue effects were seen amongst rats receiving 125 ppm (8-11 mg kg^{-1} d^{-1}) mancozeb for 90 d although it should be noted that no other tissues were investigated.

Groups of 12 male Wistar rats were fed dietary concentrations of 0, 100, 500, 750, 1130, 1690, 2530 or 3790 ppm mancozeb (70 % purity, containing ~0.29 % ETU) continuously for 12 weeks (Szepvolgyi *et al*, 1989). Mean doses of mancozeb, as a function of body weight were calculated as equivalent to 0, 7, 35, 52.5, 79.1, 118.3, 177.1, or 265.3 mg kg^{-1}. Routine cage side observations, haematological and clinico-chemical analyses including thyroid/serum iodine content and hepatic MFO function, and pathology of a range of organs were conducted.

At the top dose, prostration and hind limb paralysis were observed between weeks 2 and 6, and were fully reversed by the end of the observation period. Approximately one third of rats died within 6 weeks. Animals receiving doses equivalent to $\geq$118 mg kg^{-1} mancozeb showed decreased weight from the week 4 observation, and overall, dose-related decreases in weight gain, food consumption and food conversion efficiency were observed. Haematology findings were comparable between all groups. Hepatic cholesterol and serum cholinesterase were increased in a dose related fashion. The iodine content of the thyroid was statistically significantly reduced 80 - 90 % in all groups receiving $\geq$ 35 mg kg^{-1} mancozeb and protein-bound iodine, tested in the top three dose groups, showed dose-related decreases (by approximately 50 %) compared to control animals. Dose-related decreases in the liver-weight relative activities of aminopyrine and aniline hydroxylase gained statistical significance at 177 mg kg^{-1}. Cytochrome p-450 content of the liver was unaffected. Relative (but not absolute) organ weights were provided, of note were dose-related increases in relative liver weight and thyroid relative weight were statistically significant at doses equivalent to $\geq$52 mg kg^{-1} mancozeb approximately 1.5-fold and 3-fold respectively. The main microscopic finding was dose-related increase in the incidence and severity of thyroid follicular cell hyperplasia at $\geq$ 79.1 mg kg^{-1} mancozeb. A NOAEL for systemic toxicity of 7 mg kg^{-1} d^{-1} can be identified from this study.

The results of a preliminary study investigating the sub-chronic toxicity of mancozeb have been published (Trivedi *et al*, 1993). Groups of 30 male albino rats (strain unspecified) received daily oral gavage doses of 500, 1000 or 1500 mg kg^{-1} mancozeb suspended in peanut oil for 90 d. A control group of 30 rats received peanut oil. The composition of the mancozeb use was not described but presumably was the mancozeb described as '75 wettable powder' (with no further details on purity or composition being given), used by the same team for a later study (Kackar *et al*, 1997a). At 15, 30, 60 and 90 d, four animals from each group were killed, blood samples taken and the thyroid removed. Serum was separated from blood and T_4 measured by radioimmunoassay. The thyroid was weighed and processed for histopathology.

Treatment-related deaths (0 in controls; 5 at 500 mg kg^{-1} d^{-1}; 9 at 1000 mg kg^{-1} d^{-1}; and 15 at 1500 mg kg^{-1} d^{-1}) and signs of toxicity were observed in all of the treated groups. Signs of

toxicity included dyspnoea, diarrhoea, salivation, nasal bleeding and hind limb paralysis. Effects on body weight were not reported. The thyroid to body weight ratio was increased in a time and dose-dependent manner with a slight increase at 1500 mg kg^{-1} d^{-1} at 30 d and in all groups at 60 and 90 d. Thyroid peroxidase was reduced after 30 d dosing at 1500 mg kg^{-1} d^{-1} and then subsequently in a time-dependent manner. T_4 was reduced in all groups at day 30 and from then in a time-and dose-dependent manner after this. In rats examined at 90 d, histopathological examination revealed dose-dependent changes in the thyroid including hypertrophy and hyperplasia of the follicular cells with loss of colloid mass.

A report describes the effect of mancozeb on hepatic microsomal carboxylesterases/amidases in rats following oral dosing (Siddiqui *et al*, 1990). Groups of 5-6 male rats (strain unspecified) received 250 mg kg^{-1} d^{-1} of mancozeb (75 % pure) for 7 or 30 consecutive days as an aqueous suspension by oral gavage. Control groups were included, though their treatment was unspecified. Animals were killed 16-18 h after the final dose and hepatic microsomes prepared. Activities of hepatic microsomal carboxylesterases/amidases were assessed (spectrophotometrically) by the hydrolysis of acetylsalicylic acid (ASA), 2-acetylaminofluorine (2-AF), acetanilide (ACN), p-nitrophenylacetate (PNA), the hydrolysis of acetylcholine by cholinesterase was also assessed. Treatment with mancozeb for both 7 and 30 d was found to increase the mean hydrolysis of ASA, 2AF and ACN by around 40-60 % compared to that seen in microsomes from control rats. No effects were seen on PNA or acetylcholine hydrolysis.

In a related study, groups of male Wistar rats aged 1, 21 or 120-130 days old received either 100 or 200 mg kg^{-1} d^{-1} of mancozeb (stated to be technical grade, 95 % pure) daily for 7 consecutive days as an aqueous suspension by oral gavage (Siddiqui *et al*, 1993). Age-matched controls were also included. Different age groups were included in order to investigate the influence that age at treatment might have on response. At 18-20 h after the last dose, the activity of hepatic glutathione *S*-transferase (GST) was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate in postmitrochondrial supernatant prepared from homogenized liver. Weanling and adult rats were said to tolerate the treatment with mancozeb well but around 30-35 % mortality was apparently seen in the mancozeb-treated newborn group (no indication of an dose-response relationship) compared to 10 % in the newborn controls. In all three age groups, mancozeb treatment produced a dose-dependent increase in GST activity, around 25-35 % at the lower dose and 55-75 % at the higher dose. The response in weanling rats was slightly greater.

3.3.4.1.1.2.2 Dogs

In a study conducted according to OECD guidelines and in accordance with GLP, groups of 4 male and 4 female beagles received 0, 5.7, or 34 mg kg^{-1} d^{-1} mancozeb (88 % pure, other components not stated) using orally administered gelatin capsules for 13 weeks. A further 6 males and 6 females received 340 mg kg^{-1} d^{-1} for 16 d, but this was reduced to 204 mg kg^{-1} d^{-1} for the remainder of the study period. Investigations included pre-terminal ophthalmoscopy, blood biochemistry (including T_3, and T_4) and haematology (including bone marrow samples at necropsy) in weeks 6 and 12, pre-terminal urinalysis, organ weight analysis, and extensive macroscopic and microscopic pathology examinations (Unpublished, 1988c).

There were no mortalities. Clinical signs of toxicity were only observed at the high dose group and included vomiting, yellow faeces (probably due to the presence of the test

substance or its metabolites), pallor, emaciated appearance, subdued behaviour, convulsive episodes and collapsing. There were no clinical signs of toxicity at the lower exposure levels. During the first 2 weeks, occasional body weight losses and a marked reduction in the mean body weight gain (of around 75 % less than controls) were observed and there was a marked reduction in food consumption (about 25 % lower than control) observed in males and females at 340 mg kg^{-1} d^{-1}. Occasional body weight losses resulted in a marked overall reduction in group mean body weight gain over the study period (about 80 % lower than controls) and reduced food consumption (around 20 % lower than control) was still observed upon lowering the exposure to 204 mg kg^{-1} d^{-1}. Weight gain and food consumption were not adversely affected in other groups.

Haematology investigations in week 6 showed an increase in platelet counts in males and females at the high dose group (21 % and 26 % respectively). No other parameters appeared to be adversely affected. In week 12 in males and females at the high dose group there was a reduction in packed cell volume (24 % and 30 % respectively), haemoglobin concentration (26 % and 30 %), and erythrocyte count (28 % and 32 %). Pre-terminal bone marrow samples showed an increase in neutrophils and/or erythroid series and a concommitant decrease in the myeloid to erythroid ratio in males and females at the high dose group. Blood biochemistry investigations in week 6 showed a decrease in AST activity in males at 34 mg kg^{-1} d^{-1} and the high dose group (25 % and 28 % respectively) and females at the high dose group (23 % lower, although not attaining statistical significance). Total cholesterol levels were increased in males and females at the high dose group (62 % and 52 % greater respectively). T_4 levels were decreased in males at the high dose group (30 %), and females at 34 mg g^{-1} d^{-1} and the high dose group (40 % and 35 % respectively). In week 12 AST activity was reduced in males and females at the high dose group (11 % lower, but not statistically significant and 28 % respectively). Glucose levels were reduced in males and females at the high dose group (probably as a result of reduced food consumption). Total cholesterol levels were increased in males and females at the high dose group (82 % and 25 % greater respectively). T_4 levels were decreased in males and females at the high dose group (males 24 %, although not statistically significant, and females 56 %). There were no significant changes in urinalysis parameters (volume, pH, specific gravity).

No ophthalmic lesions were observed. At necropsy, increased relative liver weights were seen in males and females at the high dose group (39 % and 47 % respectively), and also in the thyroids (180 % and 170 %), and adrenals (31 % and 42 %). Macroscopically, enlarged spleen was seen in 1/4 males and 2/4 females at the high dose group and 1/4 females at 34 mg kg^{-1} d^{-1}. Enlarged liver was seen in 1/4 males and 1/4 females at the high dose group. Enlarged and/or dark thyroids were seen in 1/4 males at 34 mg kg^{-1} d^{-1} and 2/4 males and 3/4 females at the high dose group.

Microscopically, thyroid follicular hyperplasia was seen in males (0/4, 3/4, 2/4, 4/4) and females (1/4, 2/4, 2/4, 3/4), and an associated accumulation and pallor of colloid (males; 0/4, 4/4, 4/4, 4/4, females; 1/4, 3/4, 3/4, 4/4). Testicular immaturity and a reduced number of spermatozoa were seen in 2/4 males at 34 mg kg^{-1} d^{-1} and 2/4 males at the high dose group. Minimal to slight lymphoid hypoplasia of the mesenteric lymph nodes was seen in males (0/4, 1/4, 3/4, 2/4) and females (0/4, 0/4, 2/4, 1/4).

Overall, all of the changes seen in this study are reflective of hypothyroidism due to mancozeb. Marked toxicity is seen at the highest exposure level, the high dose group for 13

weeks. However, histopathologically, lesions attributable to mancozeb were seen at all exposure levels (e.g. the LOAEL in this 13-week dog study was 5.7 mg kg^{-1} d^{-1}).

In a study conducted according to modern protocol standards in accordance with GLP, groups of 6 male and 6 female beagles received 0, 10, 100, 1000, or 5000 ppm mancozeb (83 % pure, other components not stated) by dietary administration for 13 weeks The mean intakes of mancozeb were determined to be approximately 0, 0.3, 3, 29, and 102 mg kg^{-1} d^{-1} for males and 0, 0.3, 3, 29, and 109 mg kg^{-1} d^{-1} for females. Investigations included pre-terminal ophthalmoscopy, blood biochemistry (including T_3 and T_4) and haematology in weeks 5 and 13, organ weight analysis, and extensive macroscopic and microscopic pathology examinations (Unpublished, 1986f).

Two males and one female dog at 5000 ppm (102/109 mg kg^{-1} d^{-1}) were killed due to excessive weight loss and poor condition. Clinical signs of toxicity also seen at this exposure level were dehydration, emaciated condition, pallor, and occasional instances of no or few faeces. These were attributed to the loss of appetite at this exposure level (possibly related to palatability). Body weight loss (of around 800-1500g) was seen amongst males and females at 5000 ppm and females at 1000 ppm (29 mg kg^{-1} d^{-1}). Reduced body weight gain was noted in males at 1000 ppm (55 % lower than controls). A marked reduction in food consumption was noted in males and females at 5000 ppm (reduced by about 50 % in both sexes), and in females at 1000 ppm (by around 30 %). Body weight gain and food consumption in other groups was unaffected.

A number of erythrocyte parameters were affected (at both weeks 5 and 13) by mancozeb exposure at 1000 ppm (29 mg kg^{-1} d^{-1}) and 5000 ppm (102/109 mg kg^{-1} d^{-1}); reduced packed cell volume (haematocrit) (males and females at 5000 ppm reduced by about 24 % compared to controls), reduced haemoglobin concentration (males at 5000 ppm, about 16 %, females at 1000 and 5000 ppm by about 15 % and 22 % respectively), and reduced erythrocyte counts (males at 5000 ppm, about 18 %, females at 1000 and 5000 ppm by about 18 % and 27 % respectively). Other haematology parameters were not affected. At weeks 5 and 13, increased total cholesterol levels were seen in males at 5000 ppm (by about 80 % more than controls), and females at 1000 and 5000 ppm (about 44 % and 60 % greater than controls). Decreased alanine aminotransferase (ALT) activity was noted in weeks 5 and 13 amongst males and females at 5000 ppm (about 43 % and 38 % respectively). Decreased T_3 levels were noted only in week 5 amongst males and females at 5000 ppm (58 % and 40 % respectively) and decreased T_4 was seen in weeks 5 and 13 for males and females at 5000 ppm (about 72 % and 62 %).

Increased relative thyroid weight (by around 200 %) was seen in males and females at 5000 ppm (102/109 mg kg^{-1} d^{-1}). Other organ weight changes were not related to mancozeb exposure. No ophthalmic lesions were observed. Macroscopically, enlarged thyroid was observed and decreased thymus size in animals at 1000 ppm (29 mg kg^{-1} d^{-1}) and 5000 ppm. In addition, pallor of the visceral organs was observed in all decedent animals and amongst others receiving 5000 ppm.

Microscopically, thyroid follicular hyperplasia was seen in 6/6 males and 6/6 females at 5000 ppm. Lymphoid depletion of the cortex of the thymus was seen in all males and all females at 1000 and 5000 ppm. In males at 5000 ppm, aspermatogenesis was seen in 3/6 males, hypospermatogenesis in 2/6, and hypogenesis in 1/6. In addition, hypogenesis of the epididymides was seen in 2/6 males at this exposure level. Hypogenesis of the prostate was

noted in 2/6 males at 1000 ppm and 6/6 at 5000 ppm. In females at 5000 ppm, hypogenesis of the ovaries, and uterus was seen in 4/6 females. Increased extramedullary haematopoiesis of the spleen was noted in 2/6 males and 2/6 females at 5000 ppm, and increased pigment deposition in 1/6 females at 1000 ppm and 3/6 females at 5000 ppm. Increased extramedullary haematopoiesis of the liver was noted in 2/6 males and 2/6 females at 5000 ppm, and sinusoidal cell pigmentation in 4/6 males and 2/6 females at 5000 ppm. Pallor of the *zona fasciculata* of the adrenals was seen in 4/6 males and 3/6 females at 5000 ppm.

Overall, the major toxicological manifestations were effects on the thyroid, thymus, erythrocyte parameters, and development of reproductive organs amongst animals at 1000 and 5000 ppm (29 and 102/109 mg kg^{-1} d^{-1}). At these exposure levels there were also marked effects on food consumption and body weight gain that could be attributable to palatability. Such effects would also alter all of those tissues noted. Mancozeb, or its metabolites undoubtedly reach the thyroid, but it is difficult to determine the extent to which reduced food consumption would have influenced the profile of effects seen. Notwithstanding this uncertainty, a clear NOAEL of 100 ppm (3 mg kg^{-1} d^{-1}) was identified.

3.3.4.1.1.3 Ethylene Thiourea

3.3.4.1.1.3.1 Rats

Groups of Sprague-Dawley rats (14 per sex per group) were continuously administered ETU (99 % purity) in the diet at concentrations of 0 or 250 ppm for up to 3 months. The mean calculated doses of ETU received, as a function of body weight and corrected to the nearest whole number were 0 or 14 mg kg^{-1} d^{-1} in males and 0, or 18 mg kg^{-1} d^{-1} in females (Unpublished, 1986e).

All animals were monitored throughout for clinical signs of toxicity, body weight and food consumption. At study termination blood from 10 animals per sex per group was taken for haematological, clinical chemistry and thyroid function analysis. The animals were killed, all standard organs were weighed, macroscopic and histopathological investigations conducted on a wide range of tissue and organs. Liver samples from 6 animals per sex per group were used for analysis of mixed function oxidase (MFO) activity by both aniline hydroxylation and AP N-demethylation.

All animals survived to study termination. No clinical signs of toxicity were observed in any of the animals. Decreased mean body weights of less than 15 %, and reduced food consumption were noted in both sexes. No treatment-related ocular effects were observed. Absolute and relative liver weights were statistically significantly increased in both sexes (both by 11 % in males, and by 7 and 15 % in females). Absolute and relative thyroid weights were also statistically significantly increased in both sexes (by 80 and 84 % in males, and by 68 and 80 % in females, respectively). Platelet counts were slightly decreased in males (11 %) and serum cholesterol levels increased in both sexes (52 % in males and 23 % in females). No changes in urine analysis were detected. Serum thyroxine (T_4) levels were statistically significantly reduced by 50 % in males and 65 % in females. TSH levels were statistically significantly increased 4-fold in males and 2.5 fold in females**.** T_3 levels were statistically significantly increased in males and females by 28 and 16 %, respectively. MFO activity was decreased, but not statistically significantly, when measured by AP N-demethylation by 29 to 37 % in males only. AP N-demethylation in females and aniline hydroxylation activity in both males and females were not affected. No effect on hepatic

microsomal protein concentration was observed in animals across the exposure range. Histopathological investigation showed evidence of effects on thyroid and pituitary glands and on the liver, these being reported as; thyroid follicular cell hypertrophy or hyperplasia in both sexes; an increased presence of hypertrophied cells in the anterior pituitary in males, and hepatocellular hypertrophy of minimal severity in males and females.

Overall, the results of this study indicate that the thyroid, pituitary and the liver are the key organs affected by ETU; the effects observed include marked increases in liver weight associated with microscopic cytoplasmic changes, together with disturbances in MFO activity. Slight thyroid hyperplasia and reductions in plasma T_4 levels are also observed. As only one concentration was tested a NOAEL cannot be determined from this study.

In a dose selection study for a subsequent carcinogenicity bioassay, as part of a series of NTP studies, F/344 rats (10 per sex per group) were administered ETU (purity 99 %) in the diet at concentrations of 0, 60, 125, 250, 500 or 750 ppm for 13 weeks. Using the data regarding body weights and food consumption provided in the report, the mean intake of ETU was calculated to be 0, 3, 6, 12, 23 or 43 mg kg^{-1} d^{-1} in males and 0, 4, 8, 15, 29 or 48 mg kg^{-1} d^{-1} in females. The animals were monitored throughout for clinical signs of toxicity, body weight and food consumption. At study termination all standard organs were weighed, macroscopic investigations conducted on a wide range of tissue and organs. Histopathological examination was conducted on all animals from control and 750 ppm exposure groups (Unpublished, 1989b).

All animals survived to study termination. No clinical signs of toxicity were observed in any of the animals. The final mean body weights of male rats up to 250 ppm and female rats up to 500 ppm were comparable with controls. Relative to controls, the final mean body weights of male rats at 500 and 750 ppm and of females at 750 ppm were reduced by 10, 32 and 28 %, respectively. Food consumption levels of males and females at 500 and 750 ppm were reduced by 16 % and 25 %, and compared with controls. Consumption in other groups was unaffected.

At necropsy lesions in the thyroid and pituitary glands and the livers of animals in all exposure groups, were observed. Histopathological examination revealed focal follicular cell hyperplasia in the thyroid glands of 5-6 males at 250-750 ppm and in 1-4 females at 500 and 750 ppm. Diffuse follicular cell hyperplasia in the thyroid glands in all males and females across the exposure range was also seen. The severity of hyperplasia was dose-related, ranging from minimal to severe. Follicular cell adenomas were observed in 3, 3 and 6 males at 250, 500 and 750 ppm, respectively and in 3 and 3 females at 500 and 750 ppm, respectively. Pituitary effects, characterised as cellular vacuolisation of the Pars distalis, were observed in 6 and 7 males at 250, and 500 and in all males and females at 750 ppm. Liver effects, characterised as centrilobular cytomegaly, were observed in 7 males and all females at 750 ppm. No other abnormal pathological findings were observed.

A NOAEL cannot be identified from this study. Follicular cell hyperplasia of the thyroid gland was observed in all males and females at 60 ppm, the lowest concentration tested (equivalent to 3-4 mg kg^{-1} d^{-1}).

The effects seen on the thyroid and liver in these submitted studies are consistent with those reported in other sub-chronic dosing studies with ETU (IPCS, 1988).

3.3.4.1.1.3.2 Mice

In a dose selection study for a subsequent carcinogenicity bioassay, as part of a series of NTP studies, B6C3F1 mice (10 per sex per group) were administered ETU (purity 99 %) in the diet at concentrations of 0, 125, 250, 500, 1000 or 2000 ppm for 13 weeks. The data presented in the report indicated a mean food consumption of 75 mg diet per mouse per week. However, this value is implausible. Hence, assuming a mean body weight for males of 30 g and for females of 25 g, and assuming a mean daily food consumption of 3.6 g d^{-1} and 3.25 g d^{-1} for males and females respectively, it is estimated that the dietary intakes of ETU are equivalent to 0, 15, 30, 60, 120 or 240 mg g^{-1} d^{-1} in males and 0, 16, 32, 65, 130 or 260 mg kg^{-1} d^{-1} in females (Unpublished, 1989b).

The animals were monitored throughout for clinical signs of toxicity, body weight and food consumption. At study termination, all standard organs were weighed, macroscopic investigations conducted on a wide range of tissue and organs. Histopathological examination was conducted on all animals from control and 2000 ppm exposure groups.

A total of six males (3 controls, 1 at 250 and 2 at 500 ppm) and 2 females (at 125 ppm) died before study termination, although the causes of death were not attributed to ETU administration. No clinical signs of toxicity were observed in any of the animals. There were no treatment-induced effects on body weight. Food consumption levels were compared with controls.

No treatment-related pathological lesions were observed in any animals. However, histopathological examination revealed diffuse follicular cell hyperplasia at 500 ppm and above in males (in 7, 10 and 10 animals, respectively) and in females (in 8, 9 and 10 animals, respectively). The severity of hyperplasia was dose-related, ranging from minimal to moderate. Centrilobular hepatocyte hypertrophy was also observed at 500 ppm and above in males (in all animals) and in females in 4, 9 and 9 animals, respectively). No abnormal pathological findings were observed amongst those animals exposed to 125 or 250 ppm . No other abnormal pathological findings were observed.

A NOAEL of 250 ppm (equivalent to approximately 30 mg kg^{-1} d^{-1}) can be identified from this study. Follicular cell hyperplasia of the thyroid gland and hepatocyte hypertrophy were observed in males and females at 500 ppm (equivalent to approximately 60 mg kg^{-1} d^{-1}).

3.3.4.1.1.3.3 Dogs

In a study conducted according to OECD guidelines in accordance with GLP, groups of 4 male and 4 female beagles received 0, 10, 150, or 2000 mg/kg diet ETU (98 % pure) by dietary administration for 13 weeks. The mean intakes of ETU were determined to be approximately 0, 0.4, 6, and 66 mg kg^{-1} d^{-1} for males and 0, 0.4, 6.5, and 72 mg kg^{-1} d^{-1} for females. Investigations included pre-terminal ophthalmoscopy, blood biochemistry (including T_3, and T_4) and haematology in weeks 4, 8 and 13, organ weight analysis, and extensive macroscopic and microscopic pathology examinations (Unpublished, 1992b).

Two males at 66 mg kg^{-1} d^{-1} were killed due to poor condition in week 8. Clinical signs of toxicity amongst surviving animals at 66 mg kg^{-1} d^{-1} included pallor, subdued behaviour, and occasional blood in the faeces. There were no clinical signs of toxicity amongst other treated groups. Body weight losses were noted on occasion amongst the decedent males, otherwise

there were no ETU-related effects on body weight gain. Reduced food consumption was recorded only amongst males and females receiving 66/72 mg kg^{-1} d^{-1} (about 33 % and 20 % respectively).

A number of erythrocyte parameters were affected (at weeks 8 and 13) by ETU exposure at 6/6.5 and 66/72 mg kg^{-1} d^{-1}); reduced packed cell volume (haematocrit) was seen in males at 66 mg kg^{-1} d^{-1} in weeks 8 and 13 (15 % and 19 % lower than control), and in females at 6.5 and 72 mg kg^{-1} d^{-1} in week 8 (14 % and 17 %) and at 72 mg kg^{-1} d^{-1} in week 13 (19 %). Erythrocyte count was reduced in males at 66 mg kg^{-1} d^{-1} in weeks 8 and 13 (20 % and 25 % lower than control), and in females at 6.5 and 72 mg kg^{-1} d^{-1} in week 8 (14 % and 18 %) and at 72 mg kg^{-1} d^{-1} in week 13 (24 %). Haemoglobin levels were reduced in males at 66 mg kg^{-1} d^{-1} in weeks 8 and 13 (14 % and 19 % lower than control), and in females at 6.5 and 72 mg kg^{-1} d^{-1} in week 8 (15 % and 17 %) and at 72 mg kg^{-1} d^{-1} in week 13 (24 %). At week 13 there was an increase in reticulocyte count amongst males and females at 66/72 mg kg^{-1} d^{-1} Other haematology parameters were not affected. At weeks 8 and 13, increased total cholesterol levels were seen at week 4 in males and females at 66/72 mg kg^{-1} d^{-1} (92 % and 102 %), in weeks 8 and 13 marked increases were seen in males and females at 6/6.5, and 66/72 mg kg^{-1} d^{-1}. In contrast to the previous study dog study on mancozeb, increased alanine aminotransferase (ALT) and decreased aspartate aminotransferase (AST) were observed amongst males at 6 and 66 mg kg^{-1} d^{-1}, and increased (ALT and AST) amongst all treated groups of females but only in week 4. Hence, these findings can be considered of doubtful toxicological importance. Urinalysis measurements in weeks 4, 8, and 13 did not reveal any ETU-related effects amongst the parameters recorded (volume and specific gravity only). Decreased T_3 and T_4 levels were noted in weeks 4, 8, and 13 amongst males and females at 66/72 mg kg^{-1} d^{-1} compared to controls (in males T_3 was about 40-70 % lower, T_4 about 69-90 % lower, in females T_3 was about 4-67 % lower, T_4 about 42-90 % lower).

There was a substantial increase seen in absolute thyroid weight in males and females at 66/72 mg kg^{-1} d^{-1} (males; 22 times greater than control, females 25 times greater). Other groups were not adversely affected. Relative liver weight was also increased in males and females at 66/72 mg kg^{-1} d^{-1} (males; 18 %, females 25 %). In addition, relative adrenal weights were increased in males and females at 66/72 mg kg^{-1} d^{-1} (males; 33 %, females 42 %).

No ophthalmic lesions were observed in any ETU-exposed animals. However, macroscopically, exophthalmia was reported in 0/4, 0/4, 1/4, 2/4 males and 0/4, 0/4, 0/4, 3/4. However, this observation is of doubtful importance given the absence of any abnormalities noted in the ophthalmoscopy or any other microscopically observable lesions. With the exception of the observation of enlarged thyroids in the top dose group, there were no other macroscopic pathology abnormalities reported. Microscopically, hypertrophy of pituitary basophilia was noted in 4/4 males and 4/4 females at 66/72 mg kg^{-1} d^{-1}. Severe thyroid follicular hyperplasia was seen in 3/4 males and 4/4 females at 66/72 mg kg^{-1} d^{-1} with associated increased colloid in 1/4 males. Moderate involution of the thymus was seen in 2/4 males and 2/4 females at 66/72 mg kg^{-1} d^{-1}. There were no other treatment-related abnormalities observed.

Overall, the toxicological effects seen in this study are attributable to hypothyroidism (marked hypertrophy and hyperplasia of thyroids was observed at 66/72 mg kg^{-1} d^{-1}). As with other dietary studies on ETU and related pesticide active ingredients such as mancozeb,

it is difficult to determine the extent to which palatability and reduced food consumption may have contributed to hypothyroidism. However, in this study the reduction in food consumption was not quite as marked as other dietary studies, so it is reasonable to suggest that the majority of the effects are attributable to the direct action of ETU on the thyroid. Frank toxicity was seen at 66/72 mg kg^{-1} d^{-1}, and a clear NOAEL of 0.4 mg kg^{-1} d^{-1} was determined, although at the next exposure level of 6/6.5 mg kg^{-1} d^{-1} effects were only marginal (mainly related to anaemia).

3.3.4.1.2 Dermal

3.3.4.1.2.1 Zineb

No data were submitted or found in the literature.

3.3.4.1.2.2 Mancozeb

3.3.4.1.2.2.1 Rats

In an unpublished study conducted in accordance with OECD guidelines, conforming with GLP, groups of 10 male and 10 female Sprague-Dawley rats received 0, 10, 100, or 1000 mg kg^{-1} d^{-1} mancozeb technical (82 % pure; other components not stated) moistened with distilled water. The substance was applied repeatedly to shaved dorsal skin under a semi-occlusive dressing for 6 h d^{-1}, 5 d $week^{-1}$ for 4 weeks by the dermal route; the application site was carefully washed and dried at the end of each 6 h application period. Local irritation effects were scored in accordance with the Draize scheme. Blood samples were collected prior to termination for extensive haematology and biochemistry investigations. A range of organ weights, and comprehensive macroscopic, and microscopic pathology investigations were performed on all animals (Unpublished, 1988d).

There was one female mortality, but this was not related to treatment with mancozeb, and there were no clinical signs of toxicity amongst any treated animals. There were no effects on body weight gain or food consumption. There were incidental occurrences of slight erythema for some mancozeb-exposed animals during the study and yellow staining of the skin was observed mainly in animals receiving 1000 mg kg^{-1} d^{-1} (presumably due to the test substance).

There were no significant changes in any of the haematology or biochemistry parameters recorded including T_3, T_4 and TSH levels. There were no significant changes in organ weights (liver, kidneys, adrenals, testes, ovaries) and no macroscopic pathology abnormalities. Microscopically, minimal to slight hyperkeratosis and acanthosis were observed at treated and untreated skin sites in controls and mancozeb-treated animals; these reactions were probably related to skin-shaving rather than representing a substance-related skin irritation reaction.

Overall, this study demonstrated no adverse local skin effects or signs of systemic toxicity in rats receiving up to 1000 mg kg^{-1} d^{-1} by the dermal route for 4 weeks, and this finding is consistent with information indicating mancozeb is poorly absorbed by the dermal route of exposure.

3.3.4.1.2.2.2 Mice

The effects of repeated dermal exposure to mancozeb on ornithine decarboxylase (ODC) activity and DNA synthesis in mouse skin have been investigated (Gupta and Mehrotra, 1992). Mancozeb (technical grade, 95 % purity) was applied to the skin of female Swiss mice (4/group) in dimethylsulphoxide (DMSO) at doses of 0, 1, 2, 4 or 10 mg.animal^{-1} for 4 d at intervals of 1 d and skin ODC activity at the treatment site assessed 5 h after the last treatment. The effect of pretreatment with cycloheximide was assessed 5 h after treatment with four doses of 2 mg per animal given at 24 h intervals. A further two groups were treated with up to 5 doses of 2 mg per animal given at either 24 or 48 h intervals and the ODC activity assessed 5 h after the last dose. Repeated application of mancozeb resulted in an increase in ODC activity at 1, 2 (maximal) or 4 mg per animal but a decrease at 10 mg per animal. Pre-treatment of the mice with cycloheximide inhibited the increase in ODC activity to around 25 % of that seen in the absence of the protein synthesis inhibitor. Repeated application at either 24 or 48 h intervals initially resulted in an increase in ODC activity (about twice that seen with a single exposure) but this reduced back to the levels induced by a single exposure by the fourth application.

3.3.4.1.2.2.3 Rabbits

In an unpublished study conducted in accordance with OECD guidelines, conforming to GLP, groups of 5 male and 5 female New Zealand White rabbits received 0, 62.5, 250, or 1000 mg kg^{-1} d^{-1} mancozeb technical (84 % pure; other components not stated) moistened with distilled water. The substance was applied repeatedly to shaved dorsal skin under an occlusive dressing for 6 h d^{-1} for 21 d by the dermal route; the application site was carefully washed and dried at the end of each 6 h application period. Local irritation effects were scored in accordance with the Draize scheme. Blood samples were collected prior to termination for extensive haematology and biochemistry investigations. A range of organ weights, and comprehensive macroscopic, and microscopic pathology investigations were performed on all animals (Unpublished, 1988b).

There was one female mortality, but this was not related to treatment with mancozeb, and there were no clinical signs of toxicity amongst any treated animals. There were no local signs of skin irritation seen on visual examination, although yellow staining of the skin was observed (presumably due to the test substance). Reduced body weight gain was noted amongst males (40 % lower than controls) and females (25 % lower) receiving 1000 mg kg^{-1} d^{-1}. Food consumption was reduced only in males at 1000 mg kg^{-1} d^{-1} (around 15 % lower than controls). Weight gain and food consumption were not adversely affected in other groups. The Authors dismissed the effects at 1000 mg kg^{-1} d^{-1} as being due to impaired food consumption as a result of the ‘discomfort’ experienced by the animals. However, given that the skin responses were only very mild in nature (not visible to the naked eye, although there was some histological evidence of skin irritation), this does not seem a reasonable dismissal.

There were no significant changes in any of the haematology or biochemistry parameters recorded including T_3 and T_4 levels. There were no significant changes in organ weights (liver, kidneys, adrenals, testes, ovaries) and no macroscopic pathology abnormalities. Microscopically, minimal acanthosis and hyperkeratosis were seen in 4/5 females at 1000 mg kg^{-1} d^{-1} and 1/4 females at 250 mg kg^{-1} d^{-1}. Focal erosion was observed in 3/5 females at 1000 mg kg^{-1} d^{-1} and 1/4 females at 250 mg kg^{-1} d^{-1}. Minimal diffuse

inflammation of the superficial dermis was observed in 0/5, 3/5, 3/5, and 3/5 males and 1/5, 3/5, 4/4, and 5/5 females. There were no other significant histopathological effects observed.

In addition, at 1000 mg kg^{-1} d^{-1}, there was some indication of systemic toxicity (reduced body weight gain) and signs of local skin irritation reactions (although slight in nature). No adverse effects were observed in animals receiving 250 mg kg^{-1} d^{-1}.

3.3.4.1.2.3 Ethylene thiourea

No data were submitted.

3.3.4.1.3 Inhalation

3.3.4.1.3.1 Zineb

No data were submitted or available in the literature.

3.3.4.1.3.2 Mancozeb

3.3.4.1.3.2.1 Rats

In a study conducted in accordance with GLP, groups of 16 male and 16 female Sprague-Dawley rats were exposed, nose-only to 0, 8, 40, and 127 mg m^{-3} mancozeb (83 % pure, other components not stated) 6 h d^{-1}, 5 d $week^{-1}$ for 13 weeks followed by a 13-week recovery period. The median mass aerodynamic diameter was determined to be 3.8-4.2 µm, GSD 2.1. Extrapolation to an oral dose based on assumptions of 100 % uptake, a respiratory volume of 6 L h^{-1}, and a mean body weight of 150 g, these exposure concentrations are estimated to be equivalent to oral doses of 0, 1.9, 9.6 and 30.5 mg kg^{-1} d^{-1}. Investigations included ophthalmoscopy, haematology, biochemistry (including T_3, T_4 and TSH), a wide range of organ weights, and extensive histopathology (Unpublished 1986h).

There were 5 mortalities in total (one male and one female at 8 mg m^{-3}, on female at 40 mg m^{-3}, and one male and female at 127 mg m^{-3}); these were not related to mancozeb exposure. There were no clinical signs of toxicity. Overall, during the 13-week exposure period, group mean body weight gain was reduced by 10 % amongst males exposed to 127 mg m^{-3} compared with controls. However, there was no effect on mean body weight gain amongst females or any other mancozeb-exposed groups. A statistically significant reduction in T_4 levels (31 % lower than controls) was noted in week 13 for females exposed to 127 mg m^{-3}, and there was also a slight, but not statistically significant increase in TSH levels (6 %). Other mancozeb-exposed females and males were not adversely affected. There were no exposure-related changes in blood biochemistry or haematology parameters.

There were no significant effects on organ weights of males or females, and no exposure-related ophthalmic lesions. Microscopically, a yellow-brown granular pigment was observed in the lumen of the proximal convoluted tubules of the kidneys in the absence of any histopathological lesions; presumably this was the test substance or a metabolite and had disappeared by the end of the 13-week recovery period. Mild thyroid follicular hyperplasia was observed in 3/16 females at 127 mg m^{-3}. There were no effects on the thyroids of other groups of mancozeb-exposed females, or amongst males, and no other treatment-related

lesions in any other organs. In this study, no local signs of toxicity were seen, although systemic effects were observed particularly with respect to thyroid function at 127 mg m^{-3} (altered T_4 and TSH levels, thyroid follicular hyperplasia) and reduced body weight gain in males. No adverse effects were seen at 40 mg m^{-3}.

In a study conducted in accordance with GLP, groups of 5 male and 5 female Sprague-Dawley rats were exposed, nose-only to 0, 8, 40, and 127 mg m^{-3} mancozeb (83 % pure, other components not stated) 6 h d^{-1}, 5 d $week^{-1}$ for 4 weeks. The median mass aerodynamic diameter was determined to be 3.7-4.4 µm, GSD 2.1-2.3. Investigations were slightly more limited than the previously reported 13-week study but included haematology, biochemistry (including T_3, T_4 and TSH), lung and thyroid weights, and histopathology examination of lungs, lymph nodes, nasal turbinates, and trachea (Unpublished, 1986i).

There were 6 mortalities in total (3 control group females, one male and one female at 40 mg m^{-3}, and one female at 127 mg m^{-3}); these were not related to mancozeb exposure. There were no clinical signs of toxicity. Group mean body weight data were not presented, but it was stated that weight gain was reduced by 12 % amongst males exposed to 127 mg m^{-3} compared with controls. There were no exposure-related effects on T_3, T_4 or TSH or on other blood biochemistry or haematology parameters recorded in week 4.

There were no effects on lung or thyroid weight amongst males or females and no exposure-related macroscopic or microscopic lesions. Overall, this report indicates that the only observable local or systemic effect was reduced body weight gain of males exposed to 127 mg m^{-3} for 4 weeks. No adverse effects were seen at 40 mg m^{-3}.

3.3.4.1.3.3 Ethylene thiourea

No data were submitted.

3.3.4.2 SUMMARY OF SUB-CHRONIC STUDIES (< 90 D DURATION)

Relatively few data are available on the effects of zineb following sub-chronic exposure in animals. Some information is available from 28 d studies in rats and 90 d studies in rabbits conducted via the oral route, but none via the inhalation or dermal routes. More data are available (essentially 90 d studies) for the structurally related mancozeb and the principal metabolite of both zineb and mancozeb, ETU. Qualitatively, where comparable, the toxicity profile of all three substances is similar, with the key common finding being effects on the thyroid and liver MFO activity. In addition, mancozeb was found to induce some effects not seen with zineb but this may be due to the more thorough and extended nature of those studies available. Because the composition of the tested zineb and mancozeb is often unclear, it is difficult to say whether the effects seen are due to the parent compound, its metabolites, contamination with other substances (particularly ETU), or a combination of these. However, on balance it would seem reasonable to conclude that at least the effects seen on the thyroid and liver MFO activity induced by zineb and mancozeb are likely to be a consequence of either production of ETU by metabolism and/or contamination of the test material with ETU. Given this probable common aetiology for these effects, it would seem reasonable to compare the toxicological properties of zineb with those of mancozeb.

In both rats and rabbits treated orally with zineb, inhibition of liver MFO and hypothyroidism were observed. A NOAEL for thyroid effects could not be established (although low doses of up to 500 ppb for 5 d were without effect), with the lowest doses tested, 50 mg kg^{-1} d^{-1} in

the rat and 90 mg kg^{-1} d^{-1} in the rabbit, producing effects on the thyroid. These effects ranged from increased thyroid weight at the lowest doses, to decreased T_4 and thyroid follicular cell hypertrophy at the higher doses (rat, 1000 mg kg^{-1} d^{-1}; rabbit' 180 mg kg^{-1} d^{-1}). Regarding inhibition of hepatic MFO activity, the NOEL for this effect was determined in rats to be 60 ppm in the diet (~6 mg kg^{-1} d^{-1}). The relevance of the changes in MFO activity are unclear, but inhibition of the ability of the liver to metabolise may be of concern in relation to the clearance of xenobiotic substances from the body.

The structurally related mancozeb has been studied in rats and dogs via oral dosing. Qualitatively similar findings to those with zineb in the thyroid and liver were observed but because of the much better quality of the studies available more useful quantitative information can be derived. In rats, the range of NOAELs for these effects was 7-10 mg kg^{-1} d^{-1}. Effects on thyroid (increased weight, decreases in T_4, increases in TSH, thyroid cell hypertrophy and hyperplasia) showed an increasing severity with dose, from dose levels equivalent to 15 mg kg^{-1} d^{-1}. A reduction in liver MFO activity was observed in one study at high dose levels, with no effects seen at 250 ppm (~15-18 mg kg^{-1} d^{-1}). In addition to effects on the thyroid and liver, lesions (demyelination, Schwann cell proliferation and neurofibrillary degeneration) on the long nerves (sciatic, tibial and sural nerves) were observed in rats at ~50 mg kg^{-1} d^{-1} and above. Muscular atrophy of the hindquarters was also observed at higher (340 mg kg^{-1} d^{-1} and above) doses. Based on studies performed in rats, which included particular investigation of nervous tissue, the NOAEL for these effects was around 8-10 mg kg^{-1} d^{-1}, similar to those for effects on the thyroid and liver. In the absence of similar investigations in other species and information on the underlying mechanism, the relevance to human health of these neuropathological findings seen in rats exposed to mancozeb is uncertain, but cannot be discounted as being of no relevance.

In dogs administered mancozeb in capsules or in the diet a similar picture of thyroid effects to that seen in the rat was observed. In addition, hepatotoxicity, haematological effects (reductions in PCV, haemoglobin concentration and erythrocyte count) and effects on the testes and ovary were seen in this species. In the dietary study a clear NOAEL of 100 ppm (3 mg kg^{-1} d^{-1}) was established but a NOAEL was not established in the capsule study that used dose levels down to 6 mg kg^{-1} d^{-1}. Haematological effects were observed at dose levels of ~30 mg kg^{-1} d^{-1} and above. Effects on the testes, manifest by reduced spermatogenesis, were observed at 34 mg kg^{-1} d^{-1} and above. These effects may have either a direct effect of mancozeb on the testes or have been mediated via effects on thyroid function. Thyroid effects were seen in both studies, with histopathological changes seen at all dose levels (~from 6 mg kg^{-1} d^{-1}) in the capsule study. Given the clear NOAEL of 3 mg kg^{-1} d^{-1} in the dietary study, this may be around a threshold for these effects; however, there is also the potential for differences in toxicokinetics, and hence toxicodynamics, following dietary as opposed to bolus dosing. At the highest doses tested in the dog (102-340 mg kg^{-1} d^{-1}) signs of severe toxicity were observed with effects seen in the liver and thymus (reduced weight and lymphoid depletion of the cortex).

Toxicokinetic data indicate that ETU is formed as a primary metabolite of zineb and mancozeb. It is probable that the effects on the thyroid, at least, are related to the presence of ETU either from metabolism and/or as a contaminant of the tested materials. The toxicological effects of ETU administration have been studied in rats, mice and dogs. A consistent picture of thyroid target organ toxicity was found, with other effects probably related to malnutrition also observed. In the rat, a NOAEL could not be identified due to the presence of thyroid follicular cell hyperplasia at 3-4 mg kg^{-1} d^{-1}, the lowest doses tested. In

mice a NOAEL of 30 mg kg^{-1} d^{-1} was identified with thyroid follicular cell hyperplasia apparent at $\geq$ 60 mg kg^{-1} d^{-1}. In dogs, a NOAEL of 0.4 mg kg^{-1} d^{-1} for ETU was identified with marginal effects (mainly relating to anaemia, also seen with mancozeb) at ~6 mg kg^{-1} d^{-1} and clear signs of effects on the thyroid seen at doses around 10-fold higher. In general, therefore, the toxic effects of ETU are essentially the same qualitatively as those seen with zineb and mancozeb. However, there are quantitative differences that are probably reflective of the difference in toxicokinetics between ETU administered directly and being produced as a metabolite of another compound. Overall, though, from the information available it would seem reasonable to conclude that the effects, at least on the thyroid and possibly on haematopoesis in the dog, produced by zineb and mancozeb have a common origin via ETU. The relationship between zineb, mancozeb and ETU with respect to thyroid toxicity is discussed further in the summary of carcinogenicity.

Regarding the dermal route of exposure, no studies have been performed using zineb. Toxicokinetic data (see Section 3.2.1.2.1) indicate that there is a relatively low potential for dermal absorption of zineb and it would be predicted to be of low sub-chronic toxicity when applied directly to the skin. Consistent with this picture, conventional studies of mancozeb applied to the skin of rats and rabbits for 28 d showed reduced body weight gain as the only effect seen only in rabbits at a level of 1000 mg kg^{-1} d^{-1}, well in excess of the dose levels associated with systemic toxicity following oral dosing. No effects were seen in the rat at 1000 mg kg^{-1} d^{-1} or in the rabbit at 250 mg kg^{-1} d^{-1}.

No sub-chronic studies of repeated inhalation exposure to zineb are available. In the case of mancozeb rats exposed to 127 mg m^{-3}, 6 h d^{-1} (estimated to be equivalent to 30.5 mg kg^{-1} d^{-1}) 5 d per week for 90 d showed a decrease in T_4 levels and an increase in TSH indicating a potential for absorption, metabolism and toxicity via this route of exposure. No effects were seen at 40 mg m^{-3} (estimated to be equivalent to 9.6 mg kg^{-1} d^{-1}).

3.3.4.3 CHRONIC STUDIES (> 90 D DURATION)

3.3.4.3.1 Oral

3.3.4.3.1.1 Zineb

3.3.4.3.1.1.1 Rats

In a carcinogenicity study, as detailed in Section 3.3.6.2.1.1 (Blackwell-Smith, *et al*, 1953) groups of albino rats were fed dietary concentrations of 0, 500, 1000, 2500, 5000 or 10000 ppm Zineb (purity and composition not stated) for up to 2 years. The Authors calculated mean doses of zineb as equivalent to 0, 30, 60, 150 or 600 mg kg^{-1} d^{-1}. Signs of systemic toxicity observed were; decreased body weight gain (15 %) in top dose females only, hyperplasia of the thyroid gland in animals across all groups (including controls), severity and incidence increasing with dose, and kidney congestion, nephritis and nephrosis in top dose males and females only. No other adverse clinical observations, biochemical, haematological or pathological findings were observed. A NOAEL for systemic toxicity could not be identified from this study for this test substance due to the presence of effects on the thyroid at all dose levels.

The results of repeated oral dosing with a poorly characterised zineb preparation are described in a limited report (Ivanova *et al*, 1966). A group of 10 rats (strain and sex not stated) received 900 mg kg^{-1} (one-fifth of the acute LD_{50} determined as part of this study; see Section 3.3.1.1.1.1) of zineb prepared from a preparation containing 60 % zineb but otherwise uncharacterised. A further group of 10 rats served as controls but it was not stated whether or not they received any treatment with vehicle. The dose was apparently received repeatedly over a four-month period, although the dosing regimen was not given. It was stated that 50 % of the animals survived at least 20 doses. Although descriptions were limited, a range of observations was made including body weight, behaviour, clinical biochemistry, urinalysis and histopathological examination of liver, kidneys, lungs, GI tract, spleen and heart tissue. Apparently no changes in body weight or behaviour were observed.

There were no toxicologically significant changes in clinical chemistry parameters (though it is stressed that the report carries little detail). Histopathological examination of the stomach apparently showed 'well-advanced' hyperaemia of the mucous membrane and submucosa. Examination of the liver, kidneys and heart tissue apparently also revealed histopathological changes, described as 'degenerative and necrobiotic'. Hepatic changes (apparently most marked) included basophilia and granularity in the cytoplasm of the hepatocytes and changes in nuclear size and staining. Renal changes, less marked than those in the liver, were confined to the convoluted tubules and comprised opaque swellings of the epithelium and constriction of the lumen. In the spleen a 'large quantity of golden-yellow and rust-yellow pigment in reticular cells' was apparently observed (probably an indication of compensatory haematopoesis). There was no indication in the report of the severity or extent of the findings within these tissues, of the number of animals affected and whether or not they were seen to any extent in the controls. Furthermore, the number of doses received is unclear. Given these limitations in reporting, it is difficult to draw any meaningful conclusions from this report, other than that the liver and kidney were possible target organ tissues following oral dosing.

The effect of repeated oral administration of zineb on the thyroid and adenohypophysis of the rat has been briefly reported (Ivanonva-Chemishanska *et al*, 1975). Groups of 35 male Wistar rats received twice weekly dose of 9.6 or 960 mg kg^{-1} zineb over 4.5 months. The substance was administered as a 10 % aqueous suspension by oral gavage. A third group of 35 rats served as controls. No information is given in the report on the source or purity of the zineb used in the study. At the end of the dosing period a number of different measurements were made, though the number of rats used for each of the investigations is not always clear from the report. The thyroid and adenohypophysis were examined histopathologically. Cryostat sections were obtained from some (number not clear) thyroids and stained (presumably by enzyme histochemistry, though no details of methodology are given) for succinic dehydrogenase and cytochrome oxidase activities. Electron microscopic examinations were carried out on the thyroid tissues for four rats from each group. The rat of uptake of ^{131}I by the thyroid was assessed in ten rats from each group at 5 d after the last dose of zineb. ^{131}I was administered i.p. and 'neck counts' made at 2, 4 and 24 h afterwards. 'Thyrotropic hormone' (TTH, presumably TSH) was apparently also assessed although the methodology employed was unclear. No effects on uptake of ^{131}I, histopathology or electron micropathology were seen at 9.6 mg kg^{-1}; 'TTH' levels were raised at this dose level but given the uncertainties in the methods employed the significance of this finding in light of the lack of other changes is unclear. Mean uptake of ^{131}I at 96 mg kg^{-1} was increased (by between 40-80 % compared to controls over the time periods studied). Morphological changes in the thyroid were also observed at this dose level. The thyroid was enlarged and numerous

microfollicles were observed by histopathology, with an increase in the height of the follicular cells with nuclear vesiculation and vacuolation of the cytoplasm. An increase in succinic dehydrogenase and cytochrome oxidase activities were also observed. 'TTH' was again raised at this dose level. In general, the changes described were indicative of trophic stimulation of the thyroid. Overall, the results of this study indicate that 96 mg kg^{-1} zineb twice weekly over 4.5 months induced signs of thyroid gland stimulation, presumably as a response to compromised thyroid function. Essentially no effects were seen at 9.6 mg kg^{-1} though 'TTH' was apparently raised. This apparent no effect level should be treated with some caution due to uncertainties over the analytical technique for 'TTH' and the limited (twice weekly) dosing regimen.

In a poorly reported study, groups of rats (group size and strain not specified) received 0, 12.5 or 125 mg kg^{-1} zineb (purity of test substance, and method of administration not stated) for up to 6 months (Dinoeva, 1974). At the end of each month 5 animals from each group were killed, and histological investigations performed (extent of these unclear). No information was given regarding the findings at each time point, and there was no quantification of any of the changes claimed. There were no mortalities. Apparently, zineb-exposed animals appeared agitated. It was stated that there was body weight loss. It was also stated that there was reduced alkaline phosphatase activity and RNA content in the liver, and reduced vitamin C content in adrenals. Spleen weight was stated to be unaffected (again, no further details), although apparently there was enlargement of ‘spleen follicles’ in animals receiving 125 mg kg^{-1}. Also, at this exposure level there was ‘marked vascular dystrophy’ in the liver and kidneys. Apparently, there were no adverse effects in animals receiving 12.5 mg kg^{-1}. Overall, given the extremely limited information on procedures and results, no conclusions can be drawn from this study.

A published paper is available in which the effects on the thyroid of repeated oral exposure to zineb is available (Fytizas-Daniel, 1977). The zineb used was selected from a variety of available samples (purity range 70 - 93 %) on the basis that it contained the highest ETU content of 6 % as determined by thin layer chromatography (sample range of ETU was 0.06 - 6 %). Groups of 20 Wistar rats per sex were dosed by oral gavage with zineb (93 % zineb, 6 % ETU) as a suspension of 3 % in methyl cellulose daily for 12 months at 0, 75, 150 and 300 mg kg^{-1} d^{-1} (corresponding to doses of ETU of 0, 0.45, 0.9 and 1.8 mg kg^{-1} d^{-1}). Body weights were routinely measured and 6 animals per sex per group were killed at 2, 6 and 12 months for histological examination of the thyroid gland.

Graphically presented data indicate a dose-related increase in body weights in males, whilst in females no such finding was apparent. Body-relative thyroid weights were increased in a dose-related manner; 18 %, 76 % and 134 % at 75, 150 and 300 mg kg^{-1} d^{-1}, respectively in females and 15, 125 and 183 % in males. At 2 months, histopathology of the thyroid gland showed a dose-related increase in the occurrence of epithelial hyperplasia, colloid diminution, and distension of the follicles over the whole dose range. At 6 months, lesions were considered to be more severe in animals treated at 150 and 300 mg kg^{-1} d^{-1} with disappearance of the colloid, ‘considerable’ cell proliferation and degenerative hyperplasia. At 12 months, effects were described as more severe, with intense epithelial proliferation, formation of new follicles and 2 occurrences of adenoma. As the histopathological data were only briefly reported, it is difficult to assess the severity and incidence of the reported observations or determine dose-response information from the report.

Groups of 10 female Wistar rats were fed dietary zineb (purity 90 %, without further description) at a concentration of 0 or 1300 ppm, reported to be equivalent to 0 or 117.5 mg kg d^{-1} for 7 months (Przezdziecki *et al*, 1969). The following parameters were assessed: feed consumption; body weight; a limited serum biochemical analysis; relative liver, kidneys and spleen weights; absolute adrenal, uterus, ovaries, pituitary and thyroid weights; and microscopic examination of a limited range of organs.

No deaths occurred. Graphical data indicate that feed intake was slightly reduced throughout the exposure period and body weights appeared to be reduced during the latter half of the study but recovered somewhat by termination. Serum total protein was slightly decreased in test animals (6.9 $\pm$ 0.08 vs. 7.35 $\pm$ 0.11, g per 100 ml serum) and the proportion of gamma-globulin was increased 30 % (values of 10.5 $\pm$ 1.89 vs. 13.72 $\pm$ 1.69, %) whilst levels of albumin, alpha 1-, alpha 2- and beta globulin showed only small changes (<10 %). In treated animals, a small increase in serum sodium (12 %) was observed, but compared to control values, potassium and calcium levels were unremarkable. Parameters of white blood cell values were comparable between groups. Only small changes (<15 %) in organ weights were observed except in the case of the adrenal glands (+23 %) and the uterus (-17 %), and in all cases there was a large range of values (judging by the standard error of mean as presented). Apparently, microscopic pathology examinations showed no significant findings in the organs studied. Overall, given the lack of pathological changes and the large degree of variability, no toxicologically significant effects were apparently seen in this study. However, this outcome should be viewed with caution in light of the effects seen in other studies at similar dose levels.

In a very poorly reported study it was stated that an unspecified number of rats receiving 9800 mg kg^{-1} d^{-1}, aqueous zineb (purity not stated, containing 0.5 - 2 % ETU) 2 d per week, for up to 4.5 months showed signs of enlarged thyroid with increased enzyme activity whilst this finding was slight or absent at 980 mg kg^{-1} d^{-1} (Kaloyanova and Ivanova-Chemishanska, 1989). However, very few data or experimental details were reported and it was not possible to quantify the toxicological findings. Overall, given the lack of information, few conclusions can be drawn from this report.

3.3.4.3.1.1.2 Dogs

A brief published report of an old 1-year feeding study in dogs is also available (Blackwell-Smith, *et al*, 1953). Groups of 3 mongrel dogs were fed moistened diets containing 20, 2000 or 10000 ppm zineb (purity not specified). A group of 5 untreated animals were used as a control group. The animals were weighed weekly. Haematological analysis (erythrocyte count, haemoglobin concentration and differential white cell count) was conducted pre-treatment and at 6 and 12 months. Gross and histopathology examinations were conducted on all survivors at 12 months. The tissues examined included heart, lung, thyroid, liver, stomach, spleen, kidney, small and large intestine and the gonads. At necropsy the thyroid glands were removed and ratios of thyroid weight to body weight determined.

All animals survived to the end of the dosing period. No change in body weight was observed. No information regarding clinical signs of toxicity was reported. No treatment-related changes in haematology were observed. Increased relative thyroid weight was reported in top dose animals only (no quantification of effects was reported). Thyroid hyperplasia, of moderate severity (grade 3 on scale of 0 to 5) was observed in top-dose animals. No histopathological changes to thyroids were observed in low and mid-dosed

animals. No treatment-related effects were reported to occur in any other tissue examined. No evidence of an increase in treatment-related tumours was reported.

3.3.4.3.1.2 Mancozeb

3.3.4.3.1.2.1 Rats

In a carcinogenicity study, as detailed in Section 3.3.6.2.1.2, groups of Sprague-Dawley rats were fed dietary concentrations of 0, 25, 100 or 400 ppm mancozeb (88 % purity, remaining composition not stated) continuously for up to 104 weeks. Mean doses of mancozeb, as a function of body weight were calculated as equivalent to 0, 1, 4 or 16 mg kg^{-1} d^{-1} in males and 0, 1, 5 or 21 mg kg^{-1} d^{-1} in females. Signs of systemic toxicity observed were; decreased body weight (5-6 %) in top dose males and females, decreased serum T_4 levels in top dose females, discoloured testes in top dose males, thyroid masses in males at 100 and 400 ppm, and 'slight' thyroid follicular hyperplasia in top dose animals. No other adverse clinical observations, biochemical, haematological or pathological findings were observed. A NOAEL for systemic toxicity of 100 ppm (4-5 mg kg^{-1} d^{-1}) can be identified from this study (Unpublished, 1992d).

In a combined chronic toxicity and oncogenicity study, as detailed in Section 3.3.6.2.1.2, groups of CD rats were fed dietary concentrations of 0, 20, 60, 125 or 750 ppm mancozeb (84 % purity, containing ~1.1 % ETU) continuously for up to 2 years. Mean doses of mancozeb, as a function of body weight were calculated as equivalent to 0, 1, 2, 5 or 31 mg kg^{-1} d^{-1} in males and 0, 1, 3, 7 or 40 mg kg^{-1} d^{-1} in females. Signs of systemic toxicity observed were; diarrhoea in top dose males, decreased serum T_4 levels (16-24 %) in top dose males and females, increased TSH levels in top dose males and females (52 and 76 % respectively), enlarged thyroid glands in top dose animals, thyroid follicular cell hypertrophy and hyperplasia in top dose animals. No other adverse clinical observations, biochemical, haematological or pathological findings were observed. A NOAEL for systemic toxicity of 125 ppm (5-7 mg kg^{-1} d^{-1}) can be identified from this study (Unpublished, 1991a).

Groups of 75 male Wistar rats received daily oral gavage doses of 0, 500, 1000 or 1500 mg kg^{-1} mancozeb suspended in peanut oil for 6 d per week for 360 d (Kackar *et al*, 1997a). The mancozeb was obtained commercially and described as '75 wettable powder', with no further details on purity or composition being given. At 30, 90, 180 and 360 d, six animals from each group were killed, blood samples taken and the thyroid removed. Serum was separated from blood and T_4 measured by radioimmunoassay. The thyroid was weighed and processed for histopathology. At these same times another six rats per group received a tracer dose of ^{125}I by i.p. injection and killed 24 h later. The amount of protein bound (PB)-^{125}I present in blood was assessed as well as uptake of ^{125}I by the thyroid. Thyroid protein and peroxidase were also measured at each of these times although the report does not indicate in how many rats and whether they were from the animals already killed for other purposes or from a separate group.

Treatment-related deaths (4 in controls; 10 at 500 mg kg^{-1} d^{-1}; 15 at 1000 mg kg^{-1} d^{-1}; and 21 at 1500 mg kg^{-1} d^{-1}) and signs of toxicity were observed in all of the treated groups. Signs of toxicity included dyspnoea, diarrhoea, salivation, nasal bleeding and hind limb paralysis. Body weight gain was reduced in a dose-dependent manner such that at the end of the study weight gain was reduced by 25 % at 500 mg kg^{-1} d^{-1} and by 40 % at 1500 mg kg^{-1} d^{-1}. The thyroid to body weight ratio was increased in a time and dose-dependent manner with a slight

increase at 1500 mg kg^{-1} d^{-1} at 30 d and in all groups at 90 d onwards. Inspection of the data indicates that the increased ratio was not due simply to reduced body weight gain in the treated groups and was thus also probably due to increased absolute thyroid weight. Histopathological examination revealed dose-dependent changes in the thyroid including hypertrophy and hyperplasia of the follicular cells with loss of colloid mass at 180 and 360 d. The uptake of ^{125}I was reduced in a time and dose-dependent manner such that after 30 d of dosing a reduction was seen at 1500 mg kg^{-1} d^{-1}, at 90 d at the top two doses and in all dose groups at 360 d. Thyroid protein content was slightly reduced (by <10 %) in the top two dose groups after dosing for 180 and 360 d. Thyroid peroxidase was reduced after 30 d dosing at 1500 mg kg^{-1} d^{-1} and then subsequently in a time and dose-dependent manner. PB-^{125}I was reduced (by 20 - 40 %) at all dose levels after 180 and 360 d of dosing. Serum T_4 was reduced after 30 d dosing with 1000 and 1500 mg kg^{-1} d^{-1} and at all doses (by 30 - 55 %) at 360 d. Overall, this study demonstrated that mancozeb at dose levels of 500 mg kg^{-1} d^{-1} or more induced severe toxic effects in rats, which included deaths. Effects on thyroid function were also observed at these dose levels but because of the severe toxicity little else can be drawn from this study.

Groups of 50 male Druckerey rats received 0, 500, 1000, or 1500 mg kg^{-1} d^{-1} mancozeb (75 % pure; other components not indicated) in peanut oil by oral gavage 6 d $week^{-1}$ for 360 d (Kackar *et al*, 1997b). Body weights were recorded daily. On days 30, 90, 180 and 360, 6 animals per dose-group were killed for estimation of serum cholesterol, and testes and epididymides removed, weighed, and examined histologically. In addition, enzyme activities were measured from the testes and epididymides that had been removed and homogenised; it was unclear whether or not these were from different tissues than those selected for histology.

There was a treatment-related pattern to mortality (4/50, 10/50, 14/50 and 21/50 respectively). General signs of poor condition were observed amongst all treated animals (such as loose faeces, laboured respiration, increased salivation, 'nasal bleeding' and 'hind limb paralysis') although the incidence per group was not provided. Reduced body weight gain was observed; 21 %, 40 %, and 39 % lower than controls respectively. Serum cholesterol levels were generally increased above control levels at each of the time points attaining statistical significance at all dose levels (for example, 10 %, 29 %, and 38 % greater than control for each of the treated groups respectively at day 360). Dose-related decreases in total protein and sialic acid content of both testes and epididymides were recorded on days 180 and 360, attaining statistical significance at 1000 mg kg^{-1} d^{-1} or more. For example, total protein values on day 360 were 4 %, 9 %, and 19 % respectively lower than control in testes and 7 %, 13 %, and 15 % lower in epididymides. Sialic acid values were 4 %, 8 %, and 13 % lower in testes and 3 %, 10 %, and 15 % lower in epididymides. Similarly, there were dose-related changes in testicular and epididymal alkaline phosphatase (increased compared to control), acid phosphatase (decreased), succinate dehydrogenase (decreased), and lactate dehydrogenase (increased). On day 360, all changes at 1500 mg kg^{-1} d^{-1} were approximately 20-30 % compared to controls.

There was no change in the relative testes/epididymides weight amongst treated animals during the first 90 d although in the latter stages, a statistically-significant increase in relative testes weight and a decrease in relative epididymides weight were recorded at 1000 and 1500 mg kg^{-1} d^{-1}. At termination, testes weights were 18 % and 25 % greater than controls respectively, and epididymides weights were 18 % and 27 % lower.

Histopathologically, no abnormalities were seen in testes and epididymides of animals exposed to up to 1000 mg kg^{-1} d^{-1}. Observations at 180 and 360 d showed, at 1500 mg kg^{-1} d^{-1}, necrotic seminiferous tubules, sloughing of germinal cells, formation of giant cells and accumulation of debris in the lumen. In addition, 'damaged epithelial cells' in the epididymal tubules with a 'loss of sperm' were observed.

The increased mortality and general poor condition show that there was severe systemic toxicity amongst all treated animals. This was accompanied by decreased body weight gain, and biochemical changes. Testicular and spermatocyte development was impaired at 1000 mg kg^{-1} d^{-1} or more but the significance of these findings is unclear given the marked systemic toxicity also seen at these exposure levels and therefore, their importance with respect to male fertility is unclear.

3.3.4.3.1.2.2 Mice

In an unpublished carcinogenicity study, as detailed in Section 3.3.6.2.2.2, groups of CD-1 mice were fed dietary concentrations of 0, 30, 100 or 1000 ppm mancozeb (84 % purity, containing ~1.1 % ETU) for up to 78 weeks. Mean doses of mancozeb were calculated as equivalent to 0, 4, 13 or 131 mg kg^{-1} d^{-1} in males and 0, 5, 18 or 180 mg kg^{-1} d^{-1} in females. Signs of systemic toxicity were observed in top dose animals only and comprised reduced body weight gains (10-18 %) in males and females and decreased serum T_4 levels (up to 56 %). No other adverse clinical observations, biochemical, haematological or pathological findings were observed. A NOAEL for systemic toxicity of 100 ppm (13-18 mg kg^{-1} d^{-1}) can be identified from this study (Unpublished, 1991b).

3.3.4.3.1.2.3 Dogs

In a well-conducted study, to GLP standards, in purebred beagle dogs, 4 animals per sex per group received mancozeb (85 % purity containing 0.1 % ETU) continuously in the diet at concentrations of 0, 50, 200, 800 or 1600 ppm for 52 weeks. The mean intakes of mancozeb were 0, 2, 8, 29 or 50 mg kg^{-1} d^{-1} for males and 0, 2, 7, 29 or 54 mg kg^{-1} d^{-1} for females. Throughout the study the animals were observed for clinical signs of toxicity, behavioural changes and body weight and food consumption monitored. Neurological examinations including (but not limited to) any unusual responses with respect to body position, activity level, co-ordination of movement and gait; any unusual movement including head flicking, circling, walking backwards; sensory function and the presence or absence of reflexes, were made at 13, 28 and 52 weeks. Rectal temperature was measured at 12, 26 and 52 weeks. Ophthalmic examination was conducted at 50 weeks. Haematological parameters, clinical chemistry, thyroid hormone level analysis and urinalysis were conducted at weeks 13, 26 and 52. At 52 weeks all surviving animals were killed and standard macroscopic analysis, organ weight measurements and extensive histopathological examination conducted (Unpublished, 1990b).

Two males at 1600 ppm were killed *in extremis* at week 10 and 11. The cause of death in one animal (acute urogenital tract lesions) was probably not treatment-related. The other animal died due to chronic anaemia, which may well have been a consequence of treatment. All other animals survived to study termination. There were no clinical signs of toxicity, neurological or ophthalmic changes attributable to mancozeb observed. There were no significant changes in body weight gain or in food consumption in animals of any dose group compared with controls.

No changes in rectal temperature or urinalysis were observed, compared with controls. Haematological investigations at week 13 revealed in females at 800 and 1,600 ppm statistically significant reductions in haemoglobin (by 11 and 18 % respectively), red blood cell count (by 11 and 17 % respectively) and packed cell volume (by 11 and 15 % respectively), compared with controls. Haemoglobin levels and packed cell volume were still slightly reduced but non-statistically significantly at weeks 26 (by 9 and 10 % respectively) and 52 (by 12 and 9 % respectively) compared with controls. No other haematological changes were found in males or females of any group. Serum cholesterol levels were increased at 13, 26 and 52 weeks in females at 200 (10, 16 and 7 %), 800 (22, 50 and 52 %) and 1600 ppm (41, 54, 44 %), and in males at 800 (13, 27 and 28 %) and 1600 ppm (37, 49 and 50 %), compared with controls.

Thyroid function analysis revealed no treatment-related changes to T_3 or TSH levels in any dose group. However, T_4 levels were reduced, but not statistically significantly at 1600 ppm at 13, 26 and 52 weeks, in both males (by 25, 24 and 20 %) and females (by 34, 25 and 40 %).

At post-mortem there were no macroscopic changes or statistically significant organ weight changes that could be attributed to mancozeb at 52 weeks. Histopathological examination revealed Kupffer cell pigment deposits in liver of females at 1600 ppm, and thyroid follicular distension in animals (males and females) at 1600 ppm.

Overall, there were no toxicologically significant effects of mancozeb administration at up to 200 ppm in the diet, equivalent to 8 mg kg^{-1} for males and 7 mg kg^{-1} for female dogs respectively. At 800 ppm or more (equivalent to 29 mg kg^{-1} in males and females), there was evidence of changes related to hypothyroidism, reduced haemoglobin and blood packed cell volumes and at higher concentrations, thyroid follicular degeneration. A NOAEL for systemic effects of 200 ppm (7 to 8 mg kg^{-1} d^{-1}) was obtained.

In a study conducted according to OECD guidelines and to the principles of GLP, groups of 4 male and 4 female beagles received 0, 2.3, 22.6, or 113 mg kg^{-1} d^{-1} mancozeb (88.6 % pure, other constituents not stated) by oral (capsule) administration daily for 52 weeks. Animals receiving 113 mg kg^{-1} d^{-1} mancozeb were killed (due to poor condition) and discarded on completion of 26 weeks of treatment with no further investigations. In-life investigations included ophthalmoscopy, haematology, blood biochemistry (including T_3 and T_4), and urinalysis conducted pre-treatment, and after 24 and 50 weeks of treatment. Bone marrow samples were taken at terminal sacrifice from all animals, and there were also extensive macroscopic and microscopic examinations (Unpublished, 1991d).

One male dog receiving 113 mg kg^{-1} d^{-1} mancozeb was killed due to poor condition (considered to be related to mancozeb treatment) in week 13, and poor general condition of females led to the termination of all animals at this exposure level in week 26. Clinical signs of toxicity amongst animals at 113 mg kg^{-1} d^{-1} included emesis, pallor, yellow/green faeces, and lethargy.

Marked weight losses (occasionally 200g losses over one week) were reported amongst females receiving 113 mg kg^{-1} d^{-1}, and prior to the early termination of all animals at this exposure level mean weight losses of up to 800g.$week^{-1}$ were recorded in females. Weight losses in males at this exposure level were less marked. There was no adverse effect on body weight gain of male dogs at 22.6 mg kg^{-1} d^{-1} or less. Reduced body weight gain was noted in females at 22.6 mg kg^{-1} d^{-1} (45 % lower than controls) although females at 2.3 mg kg^{-1} d^{-1} were unaffected. Similarly, there was a reduction in food consumption at 113 mg kg^{-1} d^{-1} (at week 13, females around 82 % of control, and males ~97 %) and amongst females at 22.6 mg kg^{-1} d^{-1} (overall, 87 % of control).

There were no exposure-related ophthalmic lesions and no effects on bone marrow parameters. There were no clear exposure-related effects on haematology parameters at 2.3 and 22.6 mg kg^{-1} d^{-1}. However, at week 24, effects on erythrocyte parameters were observed in one male and one female at 113 mg kg^{-1} d^{-1} (e.g. reduced packed cell volume, haemoglobin concentration, erythrocyte and platelet counts). In addition, and showing consistency with shorter-term studies in dogs with mancozeb, there were increases in total cholesterol levels in males at 113 mg kg^{-1} d^{-1} in week 24 (81 % greater than control) and in females at 22.6 and

113 mg kg^{-1} d^{-1} in week 24 (37 % and 17 % respectively). In males, there was a slight reduction compared to controls in T_4 levels (but not T_3) at week 24 (39 % reduction at 113 mg kg^{-1} d^{-1}) and week 50 (26 % reduction at 2.3 and 22.6 mg kg^{-1} d^{-1}). A similar pattern was seen in females: Week 24, T_4 reduced by 34 % at 22.6 mg kg^{-1} d^{-1} and 41 % at 113 mg kg^{-1} d^{-1}. Week 50, T_4 reduced by 19 % at 22.6 mg kg^{-1} d^{-1}. Urinalysis parameters were unaffected by exposure to mancozeb.

On completion of 52 weeks, increased relative liver weight was noted in females at 22.6 mg kg^{-1} d^{-1} (45 % greater than control). Increased absolute thyroid weight was seen in males receiving 22.6 mg kg^{-1} d^{-1} (36 % greater than control). There were no other exposure-related organ weight effects. Macroscopically, pallor of a number of organs and yellow discolouration of GI tract contents (thought to be due to the test substance) was observed in the male at 113 mg kg^{-1} d^{-1} that was killed due to poor condition. At 52 weeks, large and dark-discoloured thyroids were reported in one male dog that had received 22.6 mg kg^{-1} d^{-1} mancozeb. Microscopically, there were no exposure-related effects.

Overall, as with other studies in dogs, the changes seen in this study are reflective of hypothyroidism due to mancozeb. Marked toxicity was seen at the highest exposure level, 113 mg kg^{-1} d^{-1}, leading to the early termination of this group on humane grounds. There was further evidence of thyrotoxicity at 22.6 mg kg^{-1} d^{-1} (as evidenced by altered T_4 levels and increased thyroid weights). No adverse effects were seen at the lowest exposure level used, 2.3 mg kg^{-1} d^{-1}.

3.3.4.3.1.3 Ethylene Thiourea

3.3.4.3.1.3.1 Rats

In a chronic study, as detailed in Section 3.3.6.2.1.3, groups of Sprague-Dawley rats were fed dietary concentrations of 0, 0.5, 2.5, 5.0 or 125 ppm ETU (96 % purity) continuously for up to 2 years. Mean doses of ETU, as a function of body weight were calculated as equivalent to 0, 0.04, 0.17, 0.37 or 8.91 mg kg^{-1} d^{-1} in males and 0, 0.05, 0.25, 0.49 or 13.57 mg kg^{-1} d^{-1} in females. Signs of systemic toxicity were observed in top dose animals only and comprised: decreased serum levels of glucose (females) and urea (males); increased serum levels of cholesterol, G-GT, bilirubin (all males only) and uric acid (females only); reduced serum T_4 levels in males and females at weeks 29 and 50 (45 and 36 % respectively); increased T_3 (22-53 %) and TSH (117 and 94 %) levels in both sexes; increased absolute thyroid weights (males only); thyroid follicular and nodular cell hyperplasia (both sexes) and hypertrophic pituitary foci (both sexes). No other adverse clinical observations, biochemical, haematological or pathological findings were observed. A NOAEL for systemic toxicity of 5 ppm (~0.4 mg kg^{-1} d^{-1}) can be identified from this study (Unpublished, 1992c).

This study is characteristic both in qualitative and quantitative terms of chronic oral dosing studies with ETU in rats (IPCS, 1988).

3.3.4.3.1.3.2 Dogs

In a well-conducted study, to GLP standards, in beagle dogs, 4 animals per sex per group received ETU (98 % purity) continuously in the diet at concentrations of 0, 5, 50 or 500 ppm for 52 weeks. The mean intakes of ETU were 0, 0.2, 2.0 or 20.1 mg kg^{-1} d^{-1} for males and 0, 0.2, 1.8 or 20.2 mg kg^{-1} d^{-1} for females. Throughout the study the animals were observed for

clinical signs of toxicity and behavioural changes, and body weight and food consumption monitored. Ophthalmic examination was conducted pre-test and at 26 and 50 weeks. Haematological parameters, clinical chemistry including thyroid hormone level analysis and urinalysis were conducted at weeks 13, 26 and 52. At 52 weeks all surviving animals were killed and standard macroscopic analysis, organ weight measurements and extensive histopathological examination conducted (Unpublished, 1991c).

Two males and 1 female at 500 ppm diet were found moribund and were killed during the treatment period (weeks 6, 17 and 7, respectively). All 3 animals presented signs including pale mucous membranes, subdued behaviour and yellow-orange stained faeces, and of weight loss, severe regenerative anaemia and a reduced T_3 or T_4 hormone levels prior to sacrifice. In addition liver necrosis was observed at necropsy. All other animals survived to study termination. Surviving males at 500 ppm sporadically presented signs of pale mucous membranes, subdued behaviour and yellow-orange stained faeces. One male at 500 ppm also showed signs of moderate anaemia (regenerative, non haemolytic) at the end of the treatment period. No treatment-related clinical signs of toxicity were seen in any animals at 5 or 50 ppm. A dose-related, but not statistically significant, reduction in body weight gain was observed in males at 50 and 500 ppm (8 and 15 %, respectively), and in females at 500 ppm (16 %). There were no significant changes in food consumption in animals of any dose group compared with controls. No ophthalmic changes were observed in any animals. The results of urinalysis showed no toxicologically significant findings.

Haematological investigations revealed a statistically significant decrease in mean haemoglobin level at weeks 13, 26 and 52 in males at 500 ppm (by 17, 14 and 22 %, respectively compared with controls), and a decrease in red blood cell count at weeks 13 and 26 in males at 500 ppm (by 17 and 13 %), respectively compared with controls.

Globulin levels were statistically significantly increased at weeks 13, 26 and 52 in animals at 500 ppm (by 19, 26 and 27 %, respectively, combined males and females) compared with controls. AST and ALT levels were increased in males at 500 ppm, but not statistically significantly, at weeks 13 and 26 (by 39 and 13 %, and by 160 and 144 %, respectively) compared with controls. AST and ALT levels were comparable with controls at week 52. Thyroid function analysis revealed no treatment-related changes to T_3 or T_4 levels in any of the surviving animals, at any sample time.

At post-mortem a dose-related and statistically significant increase in absolute thyroid weights was noted in females at 50 and 500 ppm (50 and 72 %, respectively), and in males at 500 ppm (270 %), compared with controls. There were no other organ weight changes or macroscopic changes observed which could be attributed to ETU. Histopathological examination revealed Kupffer cell pigment deposits in livers of males and females at 50 and 500 ppm, and thyroid follicular hypertrophy with colloid retention in males and females at 50 and 500 ppm.

Overall, there were no toxicologically significant effects of ETU administration at up to 5 ppm in the diet, equivalent to 0.2 mg kg^{-1} d^{-1} for males and females. At 50 ppm (equivalent to 2 mg kg^{-1} d^{-1} for males and 1.8 mg kg^{-1} d^{-1} for females respectively), there was evidence of slightly reduced body weight gain, reduced haemoglobin levels, enlarged thyroid glands with follicular hypertrophy and pigment accumulation in the liver, in males and females. At 500 ppm (equivalent to 20 mg kg^{-1} d^{-1} for males and females), there was evidence of reduced body weight gain, increased AST and ALT levels, enlarged thyroid glands with hypertrophy,

pigmentation of liver in males and females. A NOAEL for systemic effects of 5 ppm (~2 mg kg^{-1} d^{-1}) can be determined from this study.

3.3.4.3.2 Dermal

3.3.4.3.2.1 Zineb

No data were submitted or available in the literature.

3.3.4.3.2.2 Mancozeb

In a skin painting study, as detailed in Section 3.3.6.3.1.2 (Shukla *et al*, 1990), 100 mg of mancozeb (95 % purity, remaining composition not stated) in DMSO was applied to the skin of groups of 20 female Swiss mice, 3 times per week for 60 weeks. Signs of systemic toxicity observed were; reduced body weights (16 %), decreased food consumption, lethargy, scaly skin and baldness, dermal thinning and complete disappearance of the subcutaneous fat layer and keratinised skin. A NOAEL cannot be identified from this study.

3.3.4.3.2.3 Ethylene thiourea

No data were submitted.

3.3.4.3.3 Inhalation

3.3.4.3.3.1 Zineb

Groups of 10 rats (strain and sex not stated) were exposed to a dust of zineb at the following exposure chamber concentrations: 2.27, 9.9 and 47 mg m^{-3} (Ivanova *et al*, 1966). The zineb was obtained as a preparation containing 60 % active ingredient and it is unclear whether or not the cited values are for total dust or zineb. Furthermore, no information was given on particle size distribution, although samples for analysis were taken. However, no data were presented on the results of the atmospheric analysis and so it is not clear whether the concentrations cited are nominal or measured. It is likely that the exposures were whole body, although again this is not stated. The number of exposures that were administered and their duration is not stated, although the overall duration of the study was stated to be four months. Two control groups of 10 rats were also apparently used, although it is unclear whether or not these were sham exposed. Although descriptions were limited, a range of observations was made including body weight and behaviour, clinical chemistry, urinalysis and histopathological examination of liver, kidneys, lungs, GI tract, spleen and heart tissue. Notably, the thyroid was not included in the range of tissues studied, severely limiting the value of this study.

Loss of body weight (~20 %) was seen at the highest exposure concentration; weight gain was unchanged at the other exposure levels. There were no convincing changes in parameters of clinical chemistry that could be related to zineb treatment. Macro- and microscopic changes were stated to be qualitatively similar at all the exposure concentrations tested with only quantitative differences (presumably meaning in extent and severity). In the lungs a 'bluish marbling' was observed, due to the colour of the deposited test material. Histopathology apparently revealed hyperaemia and a clear thickening of the alveolar septa, within which a moderate amount of proliferative histiocytic elements and macrophages could

be seen. The cytoplasm of macrophages was full of dark-blue granules. This description appears to be typical of the response that would be expected to the deposition of a dust, possibly with some overload of the clearance mechanisms. Apparently the blue pigmentation was also found in many of the cells lining the alveoli. A large number of macrophages were also found in the peribronchial and perivascular lymph nodes. A weak proliferation of the goblet cells was observed in the bronchial epithelium with an increase in mucus secretion.

Histopathological examination of the liver apparently revealed marked degeneration of the parenchyma and hyperaemia. The changes were described as swelling, granularity and lighter staining of the hepatocytes, with eosinophilia of some cells and pyknotic nuclei. PAS staining was reduced and there were proliferative changes (not further described) of the reticulo-endothelial cells of the sinusoids. Enzyme histochemistry of liver tissue apparently showed a reduction (presumably as compared to controls) of cytochrome oxidase and succinic dehydrogenase activity in the peripheral hepatic tubules but no further details were provided. Other enzymes (e.g. LDH, ACDH) were also apparently reduced in activity. Alkaline phosphatase, acid phosphatase and non-specific esterase activities were also apparently reduced in hepatocytes. Histopathological changes in the kidney were apparently seen in the convoluted tubules, specifically as opaque swelling of the epithelium, changes in PAS-staining and in enzyme histochemistry (reductions in cytochrome oxidase and succinic dehydrogenase activity). Changes in the spleen were indicative of increased haemosiderin in the reticular cells.

Although toxic effects were described in this report, it is difficult to draw any firm conclusions given the limitations in the reporting. No information was provided on the extent or severity of the changes or of the number of animals affected. Although some indication is given that an exposure-response relationship was observed, it is difficult to judge this relationship and whether or not the changes seen, particularly at the lowest exposure concentration, were of toxicological significance. However, the Authors state that in the group that received 2.27 mg m^{-3} 'no biochemical changes were found - all that was present was some mild histo-enzymatic disturbances'. Overall, given the uncertainties within the reporting it is not possible to draw firm conclusions from this report, although qualitatively, the information provided suggests that changes typical of those associated with dust exposure and clearance were seen in the lungs and that the liver and kidney may be target tissues.

3.3.4.3.3.2 Mancozeb

Groups of 10 rats (strain unspecified) were exposed to 0, or 100 mg m^{-3} zineb for 4 months (Izmirov *et al*, 1969). No information was given on whether exposure was nose-only or whole-body, there was no information on whether zineb was in particulate form, solution or aerosol, and no information on particle/droplet size. Testes were removed, homogenised, lactate dehydrogenase (LDH) extracted and isoenzymes separated by gel electrophoresis. Enzyme activity was not recorded and it was not clear how the isoenzymes were quantified. Mancozeb had no measurable effect on the amount of LDH isoenzymes, but given the extremely limited information on procedures used it is impossible to draw any meaningful conclusions from this work.

3.3.4.3.3.3 Ethylene thiourea

No data were submitted.

3.3.4.4 SUMMARY OF CHRONIC STUDIES (>90 D DURATION)

As for sub-chronic toxicity, the data available on the toxicity of zineb following chronic dosing are limited to relatively few studies performed in rats and dogs mainly via the oral route. More information, generally of a better quality, is available for the structurally related mancozeb and the principal metabolite ETU. The toxicological profile that emerges following chronic dosing is essentially similar to that following shorter term repeat dosing with all three substances and thus comparison between them is again considered to be a reasonable approach to adopt.

Chronic oral dosing studies have been carried out on zineb in rats and dogs. These have demonstrated the thyroid to be a principal target tissue with effects also seen in the kidney at relatively high (600 mg kg^{-1} d^{-1}) doses. The studies in rats did not generally result in a robust NOAEL, with effects on the thyroid (weight increase and histopathological changes including cellular hypertrophy and hyperplasia) being seen at all doses tested (from ~75 mg kg^{-1} d^{1}). Essentially no effects were seen in one study at 9.6 mg kg^{-1} d^{-1} administered to rats twice weekly for 4.5 months. However this value should be treated with some caution because of the limitations of the dosing regimen and uncertainties over some of the experimental techniques employed. No effects were seen at 2000 ppm zineb in the diet (~50 mg kg^{-1} d^{-1}) in an older study in dogs with effects on the thyroid seen at 10000 ppm (~250 mg kg^{-1} d^{-1}).

The chronic toxicity of mancozeb with oral exposure, revealing a similar pattern of effects to those of zineb, has been studied in rats, mice and dogs, often as part of cancer bioassays. In general, all the studies are of a much better quality compared to those available for zineb. In rats, NOAELs were identified in the range 4 - 7 mg kg^{-1} d^{-1}, with signs of effects in the thyroid being seen at dose levels of ~15 mg kg^{-1} d^{-1} and above. Unfortunately, it is difficult to make direct quantitative comparisons with zineb because of the difference in the quality of the studies available and the generally higher doses of zineb used. However, from the limited information available, it would broadly appear that the same type (thyroid) and severity of effects are seen over a similar dose range for the two substances. Thus it is probably reasonable to use the mancozeb rat NOAELs as predictions of NAELs for zineb. In mice, a NOAEL of 13-18 mg kg^{-1} d^{-1} was identified in a 78 week study with mancozeb with effects seen on the thyroid at ~130 mg kg^{-1} d^{-1}, the highest dose tested. As there are no studies in mice using zineb or from shorter-term exposure with mancozeb, few comparisons can be made, although mice appear to be somewhat less responsive than rats to mancozeb (and thus probably zineb). In one (52 week) of the two dog studies available, a NOAEL of ~8 mg kg^{-1} d^{-1} for mancozeb was identified. Evidence of slight reductions in haemoglobin, red blood cell count and packed cell volume were seen at ~30 mg kg^{-1} d^{-1} and above, with effects on the thyroid (follicular distension, increased absolute weight and colloid retention) and liver (Kupffer cell pigment deposits) only seen at ~50 mg kg^{-1} d^{-1}. Comparison of these studies with the one available for zineb suggests that zineb may be less active in the dog than mancozeb, though less reliance can be placed on the older, less well-reported zineb study.

Chronic oral dosing with ETU in the rat was found to produce effects on the thyroid (increased weight, follicular and nodular cell hyperplasia) at ~10 mg kg^{-1} d^{-1}, with no effects seen at ~0.4 mg kg^{-1} d^{-1}. In dogs no effects were seen at ~0.2 mg kg^{-1} d^{-1}. Effects on the liver (Kupffer cell pigment deposits) and thyroid (increased weight, follicular cell hypertrophy with colloid retention) were seen at ~2 mg kg^{-1} d^{-1} with increased severity at ~20 mg $kg^{-1}d^{-1}$.

Overall, the effects of chronic oral dosing with zineb, mancozeb and ETU are qualitatively similar to those seen after shorter term dosing, with effects on the thyroid and liver most prominent. The effects on the thyroid induced by zineb and mancozeb are consistent with those known to be produced by ETU as exemplified by the submitted chronic studies on ETU in rats and dogs. It is difficult to make direct comparisons on a quantitative basis between zineb and mancozeb with the relative qualities of the databases available. However, taking into account the similarities in toxicokinetics between the substances, it would seem reasonable to use the information available for mancozeb where data are limited for zineb, particularly where a common aetiology for effects is likely to exist. Such a read across would suggest NAELs for zineb for oral dosing of around 4-10 mg kg^{-1} d^{-1} for the rat and dog, with a slightly higher value for the mouse. In contrast, ETU appears to be more potent than mancozeb (and thus probably zineb) with NOAELs and LOAELs up to around 10-fold lower. This difference, though, is perhaps to be expected on the basis that comparison is being made between studies involving the direct application of ETU with those where ETU is either generated principally as a metabolite and/or may be present as a low level contaminant. What is critical is that the effects seen are basically similar across all three substances, indicating that ETU is probably the main factor in the induction of effects, at least on the thyroid, by zineb and mancozeb.

Chronic dosing via other routes of exposure has been poorly studied. No dermal dosing studies are available for zineb or ETU and no useful conclusions can be drawn from the only study available for mancozeb. However, given the low bioavailability of zineb via this route, it would be predicted to be of low chronic dose toxicity when administered to the skin (increasing the uptake via the skin would undoubtedly alter this profile).

With respect to inhalation exposure, the only study available on zineb indicates that the liver and kidney may be target tissues. However, since the thyroid was not studied and there were limitations in the reporting of the study it is difficult to draw any firm quantitative conclusions. No useful studies were available on mancozeb or ETU following inhalation exposure.

3.3.4.5 HUMAN DATA (REPEATED EXPOSURES)

Nerve conduction velocities were measured using skin surface electrodes to determine peripheral and autonomic nerve function in a cross-sectional study of flower-bulb workers (Ruitjen *et al,* 1994). From a total population of 922 registered male farmers only 501 volunteered (54 % response rate) and from these 501 a study population of 131 was selected. Workers were included on the basis that they had worked at least 10 years in this industry; exclusion criteria included medical conditions such as diabetes, ‘frequent’ alcohol consumption (no further details). A group of 67 referents were selected from the same geographic area and matched against the study population by age, alcohol consumption, education, physical work activity and socioeconomic status. Referents did not include anyone from the agricultural industry, anyone who had been occupationally exposed to ‘neurotoxic’ substances, or who had been unemployed for more than one year. Occupational exposure was estimated to include zineb, maneb, captan, formaldehyde, organic mercury compounds, and captafol but was not quantified in any way. Autonomic nerve function was assessed by using a cardiotachogram to measure variation in heart rate at rest (resting arrhythmia), during deep breathing (forced respiratory sinus arrhythmia) and in response to isometric muscle contraction (muscle-heart reflex). Nerve conduction velocity, response

amplitudes and latency were measured for median and ulnar nerves in the forearm and hand, and from the peroneal and sural nerves in the leg.

Slight differences in autonomic nerve function were recorded in the study population (lower resting arrhythmia, forced respiratory sinus arrhythmia, and muscle heart-reflex). For example, mean resting arrhythmia in the referent group was 1.9 $^{+}$/- 0.9 beats min^{-1} and in exposed workers 1.6 $^{+}$/- 0.9 beats min^{-1}. A slightly lower nerve conduction velocity was reported in motor and sensory nerves and was more pronounced in the legs than arms. For instance, the mean nerve conduction velocities (m s^{-1}, $^{+}$/- SD) were for referents and exposed workers respectively: median nerve 58.8 $^{+}$/- 3.3, 57.6 $^{+}$/- 3.6, ulnar nerve 58.1 $^{+}$/ 4.9, 57.8 $^{+}$/- 4.3, peroneal nerve 45.4 $^{+}$/- 3.1, 43.6 $^{+}$/- 3.0, sural nerve 38.9 $^{+}$/- 2.4, 38.2 $^{+}$/- 2.8. Although statistical significance was attained for many of these parameters there was considerable inter-individual variation. A self-administered questionnaire indicated that there was no increase in symptoms such as numbness and tingling, or weakness of hands or feet.

The values recorded demonstrate a very slight difference in measured peripheral and autonomic nerve conduction velocities amongst this group of workers compared to controls and the significance to human health is unclear given the wide variation that would normally be expected in these parameters amongst the general population. In addition, the clinical significance is unclear given the absence of any overt signs of impairment to human health. The extent of exposure to zineb (or any of the other substances mentioned in the report) has not been quantified, although this study has been conducted with workers exposed under modern conditions. Furthermore, it is difficult to reliably attribute any differences seen in this study to zineb given the potential for mixed exposures. It is also plausible that the changes could have a non-chemical aetiology for instance due to the physical nature of the work. Overall, the full significance of these differences is unclear and they cannot be reliably attributed to zineb.

A study has been carried out to investigate the immune status of workers exposed occupationally to mancozeb (Colosio, *et al*, 1996). The study was conducted at a factory in which ‘technical grade’ dithiocarbamates were produced during different time periods. During the study mancozeb had been in production for 30 d; no information was given on possible previous exposures of workers to mancozeb or other substances, though it is stated that production of mancozeb entailed the use of metal ashes, CS_2, aliphatic amines and catalyzers. The study population consisted of 14 healthy male subjects who had been employed in the production of dithiocarbamates for 5-10 years. The terms by which a healthy status was ascertained are not reported. A further 13 workers who worked in the same factory but who were 'not exposed' to chemical substances served as controls. The location and nature of work of the control workers are not reported. It was stated that all subjects lived in the same geographic area and that the groups did not differ with respect to age, dietary habits, alcohol and drug intake and smoking habits. However, no details are given within the report of how these characteristics were determined or actual comparative data presented. It was further stated that on entry to the study clinical and laboratory parameters (specified as peripheral blood count, liver and kidney function tests) did not differ between groups. No individual data are presented.

Airborne dust concentrations, containing an unspecified level of mancozeb (other components not stated) were measured using personal samplers over the first 4 h of a shift. Median levels of respirable dust were of 1.9 mg m^{-3} (SD= 2 mg m^{-3}, range 0.13-9.0 mg m^{-3}). No information on the dust concentrations in non-exposed workers was reported.

Pre- and post-shift blood and urine samples were collected for analysis from workers on day 3 of the working week and from controls on the same morning. All analyses were initiated within 4 h of sampling. Blood and urine were analysed for CS_2 levels. Blood samples were also analysed for the presence of ETU-haemoglobin adducts.

Peripheral blood mononuclear cells (PBMCs) were separated from the blood samples and assayed in cultures both with and without a range of different mitogenic stimuli and with ^{3}H-thymidine, in order to assess for cytokine (IL1 and IL2) production, lymphocyte proliferation, population classes and polyclonal immunoglobulin (IgG and IgM) production. Serum samples were also assessed for the content of polyclonal immunoglobulin (IgG, IgA and IgM), complement C3 and C4, and for the presence of rheumatoid factor and non-organ specific serum antibodies. All of the above assays were conducted using specific enzyme linked immunosorbent assays (ELISAs).

In exposed workers levels of CS_2 in blood and urine samples collected both pre- and post - shift were equivalent (median blood levels of CS_2 = 2.3-3.6 $\mu g\ l^{-1}$, range 0.02-11.2 $\mu g\ l^{-1}$, and median urine levels of CS_2 = 2.3 $\mu g\ l^{-1}$, range 0.02-77.9 $\mu g\ l^{-1}$). These levels are 10 times higher than those measured in non-exposed controls (median blood levels of CS_2 = 0.22 $\mu g\ l^{-1}$, range 0.1-0.33 $\mu g\ l^{-1}$, and median urine levels of CS_2 =0.17 $\mu g\ l^{-1}$ range 0.09-0.28 $\mu g\ l^{-1}$). The relationship of these levels to mancozeb exposure is uncertain as CS_2 was also stated to be used itself in the production of mancozeb (the occupation of the workers being assessed).

ETU-haemoglobin adducts were detected in 40 % of the exposed workers, with levels in the blood samples ranging from 0.5 to 1.42 pmol ETU/ mgHb. No information regarding levels in the non-exposed workers are reported. The significance of this finding as an indicator of mancozeb exposure and absorption is uncertain; as no information was presented linking exposure measurements with adduct formation.

Lymphocyte function analysis showed that regardless of the T-cell mitogen stimuli used (phytohaemaglutinin, anti-CD3 monoclonal antibody, and phorbol-myristate acetate) increases in lymphocyte proliferative responses were observed. When the raw data were expressed graphically, lymphocytes from workers showed levels of ^{3}H-thymidine uptake in the presence of mitogenic stimulation to be greater by approximately 25 % compared to those from non-exposed workers. When expressed as a ratio of ^{3}H-thymidine uptake in the presence of mitogenic stimulation to that seen in non-stimulated cultures ('stimulator index'), proliferative responses were approximately 2-fold greater, with statistical significance, in exposed workers compared to non-exposed workers. Mitogen stimulated IL2 production was approximately 2-fold greater in exposed workers compared to controls, however, subject variability reduced the statistical significance of this result. The Authors reported that spontaneous production of IL1 and IL2 was similar between exposed and non-exposed subjects. No changes in the other parameters of immune function assessed were observed.

The data show slight increases in lymphocyte proliferative response and IL2 production, effects associated with cell-mediated immune function, in mancozeb exposed workers. The sub-group of T lymphocytes affected was not determined, therefore the significance of the response in terms of immune status in unknown. In addition, the interpretation of these findings is confounded by potential exposure of workers to other chemicals during the production of mancozeb, and the absence of information regarding previous chemical

exposures. Furthermore, the biological significance of this finding is unclear, particularly as no clinical signs of immunologically mediated disease were detected in any of the exposed workers.

Levels in blood of thyroid hormone and ETU were measured in seasonal workers exposed to EBDC fungicides during crop spraying (Steenland, *et al*, 1997)**.** Exposure concentrations of these chemicals to which the workers were potentially exposed were not measured. Instead, three population groups were selected for investigation based on their 'likely' exposure: highly exposed crop sprayers (n = 49); low to moderately exposed landowners (n = 14); and an unexposed control group of construction workers (n = 31).

Sprayers did not wear personal protective equipment (PPE) and were exposed to unspecified levels of EBDCs (specific nature of the EBDC was not determined, although mancozeb is cited as present in a typical pesticide mix) during the preparation, mixing, barrel filling and application of a pesticide solution. The pesticide solution also contained organophosphates (chlorpyrifos or methamidophos), foliar nutrients, growth regulators and on some occasions other fungicides (captan). Landowners were full-time farmers and were 'likely' to have been present during the application of pesticide and to have been exposed to a lower level of EBDC than the sprayers.

All individuals completed a questionnaire with respect to their age, smoking habits and alcohol consumption. No other parameters of health status or demography were reported. The average age and percentage of smokers in the test populations were; 26 years and 70 % smokers for sprayers, 32 years and 50 % smokers for landowners and 22 years and 67 % smokers for unexposed controls.

Urine samples, collected on the morning of the day following exposure, were analysed for ETU by HPLC analysis. No details of the time to analysis are reported. Blood samples, collected on the morning of the day of exposure were analysed for TSH and T_4.

It is reported that most subjects contributed both urine and blood; however, some subjects contributed only one or the other (no subject contributed more than one sample of either blood or urine). Some blood samples (8 exposed and 21 non-exposed) were delayed in transit and were in an inappropriate condition for hormone analysis, but were still used for subsequent cytogenetic analysis (see Section 3.3.5.2.3.2). Subsequently 14 additional unexposed controls were recruited and blood samples taken for thyroid hormone analysis only. No details of the age, smoking habits, health status or demography were reported for these extra control subjects.

ETU analysis was conducted on urine samples from; 38/49 sprayers, 12/14 landowners and 31/31 unexposed controls. Thyroid hormone analysis was conducted on blood samples from; 32/49 sprayers, 11/14 landowners and 24/31 unexposed controls. All non-exposed control subjects, 34 % of sprayers and 50 % of landowners had levels of ETU below the limit of detection of the analysis (10 ppb). For the purposes of calculating group mean ETU levels in blood these exposed individuals were assigned an ETU level of half the limit of detection (i.e. 5 ppb). Mean urinary levels of ETU (ppb) were subsequently estimated in exposed individuals and found to be elevated in sprayers (58 ± 26) and in landowners (12 ± 3). No individual data were reported. All mean values measured of TSH or T_4 in samples from exposed individuals (sprayers and land owners) and in the non-exposed controls were within clinical control ranges. Although no differences in thyroid hormone levels between exposed

and non-exposed workers were observed in this study, few useful conclusions can be drawn given the limitations in the study design and reporting.

A cross-sectional study of 137 workers in the fruit processing industry was conducted (Kaskevich *et al*, 1981). It was not clearly indicated whether this was a study of a population from a single or several factories. The airborne concentration of zineb was stated to be greater than 0.5 mg m^{-3}. It was unclear if this was meant to be an 8 h TWA, whether this was from personal monitoring, how much greater, or if there were any other chemical exposures for these workers. Comparisons were made against a group matched for age and sex who were 'not exposed to occupational toxins', but it is not clear if any other factors were taken into consideration such as smoking, drinking, other demographic characteristics, or even the type of employment. A number of adverse health conditions were claimed (including respiratory tract irritation, chest pain, headache, increased liver disease, nervous system disorders, altered menstruation, genito-urinary inflammation) but it was unclear how such information had been gathered. Overall, given the extremely limited descriptions of the procedures used, and the considerable uncertainty on exposure conditions, no conclusions can be drawn from this study.

A cross-sectional study of 41 workers (occupation unclear) was conducted (Gerasimchuk *et al*, 1975). No information was given on exposure conditions, the control group or of methods used. Some changes in pulmonary function (such as ventilation rate, and vital capacity) were claimed but given the extremely poor level of information provided no conclusions can be drawn from this report.

Three Approval Holders responded to enquiries regarding the incidence of adverse human health effects of zineb (Unpublished, 1999a,b,c). No incidents have been reported in recent years. One Approval Holder was aware of a small number of reports of skin irritation in operators applying antifouling products containing zineb occurring approximately 20 years ago. However, these were attributed to the presence of TBTO in the antifouling products used.

The main manufacturer of zineb, reports that both technical zineb and products containing it have been manufactured in its Spanish plant since 1963 (Unpublished, 1999d). This plant has experienced no adverse effect associated with exposure to zineb. Between 6 and 15 workers are involved with the process.

No incidents associated with zineb- or mancozeb-containing antifouling products were reported to the Pesticide Incidents Appraisal Panel between 1989 and 1999.

The Belfast and Edinburgh Centres of the National Poisons Information Service have reported that they have received no enquiries concerning zineb in the periods from 1996 onwards and 1998 onwards, respectively (Unpublished, 1999e and f).

3.3.4.6 SUMMARY OF HUMAN DATA (REPEATED EXPOSURES)

No studies of workers exposed only to zineb or mancozeb are available.

In groups of flower bulb workers, slight differences were seen in measurements of nerve conduction velocities, however, the clinical significance of these findings is uncertain. It is not possible to draw any conclusions on the role zineb may have played.

In one study of workers exposed to a variety of pesticides, typically including mancozeb, a proportion of crop sprayers showed a slightly greater blood level of ETU compared to lesser exposed farm owners and non-exposed controls. No effects on serum levels of TSH and T_4 were detected in the potentially exposed workers. Supporting data are available from a study of workers involved in the production of mancozeb, where ETU-haemoglobin adducts were found in 40 % of workers. No other information on ETU-adducts following exposure to EBDCs is available. However, this finding is consistent with the observation from studies in animals of the metabolism of EBDCs to ETU.

Workers exposed to mancozeb and a variety of other chemicals including CS_2 showed a statistically significant increase in lymphocyte proliferative response and IL2 production. Due to inadequacies in exposure assessment in regard of other chemicals and previous exposures, and in light of the fact that no clinical signs of immunologically mediated disease were detected, the toxicological significance of this finding is unclear.

3.3.5 GENOTOXICITY

3.3.5.1 *IN VITRO* STUDIES

3.3.5.1.1 Studies in bacteria

3.3.5.1.1.1 Zineb

In a study conducted to OECD guidelines and GLP, zineb (91.5 % purity, remaining composition not stated) was tested for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, both in the presence and absence of Aroclor-induced rat liver S9 at concentrations up to 5000 μg per plate. The vehicle used in this study was distilled water containing sodium lauryl sulphate (SLS); this was reportedly to prevent alteration and degradation of the compound that may occur in organic solvents. Triplicate plating was used and the test was repeated. Zineb was not mutagenic under any of the test conditions employed. Bacterial toxicity was observed at 5000 μg per plate with S9 and at $\geq$1500 μg per plate without S9. Positive control substances used produced appropriate responses (Unpublished, 1985a).

Zineb S65 (purity and composition not reported) in dimethylsulfoxide (DMSO), was tested for mutagenicity in *S. typhimurium* strains TA102 and TA104, at up to 5000 μg per plate, both in the presence and absence of Aroclor-induced rat liver S9 (Franekic *et al*, 1994). Triplicate plating was used. Zineb S65 was not mutagenic under any of the conditions employed. Bacterial toxicity was apparent at concentrations of $\geq$ 500 μg per plate both with and without S9. The positive control substances used produced appropriate responses.

In another study zineb (reported as 'pure') in DMSO, at concentrations up to 300 μg per plate were tested in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, both in the absence and presence of Aroclor-induced rat liver S9 (Shiau *et al*, 1980). A ‘slight’ but not significant (less than a two fold) increase in the number of revertants was reported in *S. typhimurium* strain TA1535 only in the absence of metabolic activation at cytotoxic

concentrations (≥ 10 µg per plate). No increases in revertant counts were observed in any other tester strain either in the absence or presence of metabolic activation.

Zineb (technical grade of 96 - 99 % purity) in DMSO at concentrations of between 10 and 1500 µg per plate did not induce an increase in revertant counts in S. *typhimurium* strains TA98, TA100, TA1535 and TA1537, either in the absence or presence of phenobarbital-induced rat liver S9 (De Lorenzo *et al*, 1978). Positive controls gave appropriate responses. No information regarding cytotoxicity was reported.

In a published report on the mutagenicity testing of over 200 pesticides, zineb (purity not reported) in DMSO at test concentrations of up to 5000 µg per plate was reported to not increase the number of revertant colonies in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 or in *Escherichia coli* strain WP2 *hcr* (Moriya *et al*, 1983). No information regarding cytotoxicity was reported.

In a non-standard published study zineb (reported as 'pure') in DMSO was tested at concentrations up to 300 µg per ml, in a battery of wild-type and repair-deficient *Bacillus subtilis* mutants, both in the absence and presence of Aroclor-induced rat liver S9, for its DNA-damaging ability (spot test and DNA-damaging test), potential mutagenicity and for effects on fractional survival (Shiau *et al*, 1980). In the spot-test zineb gave positive results, concentrations of ≥ 50 µg per ml both in the presence or absence S9 resulting in a > 1 mm inhibition zone, although the zone was markedly reduced in the presence of S9. DNA damage was reported in 5 of the 15 tester strains in the absence of metabolic activation at cytotoxic (changes in optical density of culture media) concentrations ≥ 50 µg per ml). With regard to cytotoxicity (measured as an increased inhibition radius) a dose-responsive reduction in fractional survival (30 to 100 %) was observed from 100 µg in the 2 most sensitive mutant strains tested. A 50 % reduction was also observed in the wild-type at 300 µg. No quantitation of the extent of DNA-damage compared with negative controls were reported, therefore no firm conclusions can be drawn from this study.

3.3.5.1.1.2 Mancozeb

In an unpublished study, mancozeb (88 % purity, other components not stated) at concentrations of up to 250 µg per plate was tested in S. *typhimurium* strains TA98, TA100, TA1535 and TA1537, both in the presence and absence of Aroclor-induced rat liver S9. No increase in the number of revertant colonies was observed in any test strain under any of the test conditions used. Cytotoxicity in each bacterial strain was observed at the highest test concentration. Positive and negative controls gave responses in the expected ranges (Unpublished, 1984b).

Mancozeb (technical grade of 96 - 99 % purity) in DMSO at concentrations of between 10 and 1500 µg per plate did not induce an increase in revertant counts in S. *typhimurium* strains TA98, TA100, TA1535 and TA1537, either in the absence or presence of phenobarbital-induced rat liver S9 (De Lorenzo *et al*, 1978). Positive controls gave appropriate responses. No information regarding cytotoxicity was reported.

In a published report on the mutagenicity testing of over 200 pesticides, mancozeb (purity not reported) in DMSO at test concentrations of up to 5000 µg per plate was reported not to increase the number of revertant colonies in *S. typhimurium* strains TA98, TA100, TA1535,

TA1537 and TA1538 or in *Escherichia coli* strain WP2 *hcr* (Moriya *et al*, 1983). No information regarding cytotoxicity was reported.

3.3.5.1.1.3 Ethylene thiourea

It is reported that ETU has been extensively tested in numerous studies with *S. typhimurium, E. coli and B. subtilis,* both in the presence and absence of exogenous metabolic activation, generally giving negative results. There have been isolated positive findings in *S. typhimurium* strain TA1535. Overall the weight of evidence available indicates that ETU is not mutagenic in bacteria (Unpublished 1989b, IPCS 1988).

3.3.5.1.1.4 Summary of studies in bacteria

Overall, the available data indicate that zineb is not mutagenic to bacterial cells. This is supported by data for both the structurally related substance mancozeb and the principal metabolite ETU, which also are not mutagenic in bacterial test systems.

3.3.5.1.2 Studies In Yeast

3.3.5.1.2.1 Zineb

Zineb S-65 (purity and composition not reported) has been tested for its ability to produce mitotic gene conversion in *Saccharomyces cerevisiae* strain D61.M at the *cyh* locus (Franekic *et al*, 1994). Zineb at concentrations of up to 50 μg ml^{-1} was incubated with *S. cerevisiae* cells using 2 different protocols. One series of cultures was incubated for 6 h at 28 ^{o}C and another series for 4 h at 28 ^{o}C, and then placed on ice for 16 h followed by a further 4 h at 28 ^{o}C. Cells from both incubation procedures were then cultivated and counts of survivors conducted on day 3 and of revertant colonies on days 6-7. Cytotoxicity was observed in the constantly incubated cultures from 100 μg ml^{-1} (49 % cell survival) and in the iced incubation cultures from 50 μg ml^{-1} (22 % cell survival). The positive control substances used produced appropriate responses. No evidence of a treatment-related increase in the number of resistant colonies was observed with any concentration of zineb under either incubation protocol, thus indicating that zineb did not cause aneuploidy. A dose-related increase in chromosome malsegregation was reported with both the constant (up to 8 x 10^{-6} malsegregants compared with 0.5 x 10^{-6} in controls) and iced incubation cultures (up to 12 x 10^{-6} malsegregants compared with 0.5 x 10^{-6} in controls). The significance of these results to mammalian genotoxicity is unclear.

3.3.5.1.2.2 Mancozeb

A commercial preparation of mancozeb (purity not reported) induced an increase in the number of convertants per 10^{6} survivors at the ade2 loci (70 compared with 21 in controls) and at the trp5 loci (48 compared with 8 in controls) in *S. cerevisiae* (D4 strain) only at cytotoxic concentrations (5000 and 1000 ppm respectively), which resulted in only a 27 % cell survival (Siebert *et al*, 1970).

3.3.5.1.2.3 Ethylene thiourea

A study in *S. cerevisiae* is reported to indicate that ETU has a potential to induce mitotic aneuploidy, gene conversion and DNA damage (Unpublished, 1989b).

3.3.5.1.2.4 Summary of studies in yeast

Overall the data available indicate that zineb does not cause aneuploidy, but may induce a mutagenic response in yeast at cytotoxic concentrations. A similar profile of response is also presented for both the structurally related substance mancozeb and the common metabolite ETU. The significance of these results to mammalian genotoxicity is unclear.

3.3.5.1.3 Studies In Mammalian Cells

3.3.5.1.3.1 Zineb

In a chromosome aberration study duplicate samples of Chinese hamster ovary (CHO) cells were incubated with concentrations of between 0.16 and 10 μg ml^{-1} zineb (91.5 % purity, other component not stated) in sterile water plus sodium dodecylsulphate (SDS) both in the presence and absence of Aroclor-induced rat liver. CHO cells were incubated with zineb for 2 h in the presence of metabolic activation or for 20 h in the absence of metabolic activation. Based on a 50 % reduction in the mitotic index, zineb produced a cytotoxic effect at a concentration of 10 μg ml^{-1} in the absence of S9. No reduction in mitotic index was observed either at lower test concentrations in the absence of S9 or at any concentrations in the presence of S9. No change in the proportion of aberrant metaphases (excluding gaps) compared with concurrent vehicle controls was observed at any concentration of zineb, either in the absence or presence of S9. The positive control substances used produced appropriate responses. The results indicate that zineb does not induce an increase in the incidence of chromosomal aberrations either in the presence or absence of an exogenous metabolic activation system, at up to cytotoxic concentrations S9 (Unpublished, 1985b).

In an *in vitro* mammalian cell gene mutation assay, conducted to GLP standards, replicate samples of mouse lymphoma L5178Y $TK^{+/-}$ cells were exposed to 0 - 10 μg ml^{-1} zineb (purity not reported), for 3 h both in the presence and absence of exogenous metabolic activation. Cell counts were conducted following culture periods of 24 and 48 h. A dose-related increase in cytotoxicity was observed from 0.625 μg ml^{-1} and precipitation was seen at 10 μg ml^{-1}. No increase in mutation frequency was observed at any test concentration. Positive control substances used produced appropriate responses. Zineb produced no indication of a mutagenic effect, with or without metabolic activation, in this assay (Unpublished, 1985c).

Zineb has also been tested in an *in vitro* assay to measure unscheduled DNA synthesis conducted to GLP standards. Cultured human epithelioid (HeLa S3) cells were incubated for 3 h, with concentrations of 2.5 - 5120 μg ml^{-1} zineb (91.5 % purity) in an aqueous solutions containing SDS, both in the presence and absence of exogenous metabolic activation. In the unscheduled DNA synthesis (UDS) part of the study the cell suspensions were incubated with ^{3}H-thymidine and assessed by autoradiography. A duplicate experiment was conducted. No information regarding cytotoxicity or cell viability was reported. In both experiments, both in the presence and absence of metabolic activation (Aroclor-induced rat liver S9), it was reported that concentrations of $\geq$ 160 μg ml^{-1} zineb, resulted in excessive adherence of insoluble residues to the cells which obscured the nuclei, consequently results were only obtained for the 6 test concentrations up to 80 μg ml^{-1}. At concentrations up to 80 μg ml^{-1} no increase in the mean nuclear grain count per cell compared with concurrent negative controls was observed. The positive control substances used produced appropriate responses.

Overall, results indicate that zineb did not induce DNA repair in this assay (Unpublished, 1985d).

3.3.5.1.3.2 Mancozeb

A good quality study, performed to GLP standards, is available investigating the ability of mancozeb to produce gene mutations on the hprt locus of cultured mammalian (CHO) cells *in vitro* both in the presence and absence of Aroclor-induced rat or mouse liver S9. Cells were incubated with concentrations of up to 45 μg ml^{-1} mancozeb (88 % purity, remaining composition not stated) in water for 5 h in the presence of S9 and 18 to 20 h in the absence of S9. After treatment cells were grown for 8 d to permit expression of the mutation and then cultured in order to select for the HGPRT mutants. After incubation for approximately 7 d the mutant colonies were counted. No increase in the mutation frequency was observed compared with both concurrent and historical negative (solvent and untreated) controls in either the presence of rat or mouse S9 (at up to 45 or 16 μg ml^{-1} respectively) or in the absence of S9 (at up to 15 μg ml^{-1}). Treatments with concentrations of 0.25 to 45 μg m^{-1} produced a dose-related reduction in cell survival of <100 to >5 %, relative to solvent controls. Positive controls gave appropriate responses. Overall, the results indicate that under the conditions of this assay that mancozeb does not induce gene mutations at the hprt locus in CHO cells (Unpublished, 1985e).

A good quality *in vitro* UDS study in cultured rat hepatocytes, performed to GLP standards, is available. Cells were incubated in the presence of concentrations of between 0.025 and 1000 μg ml^{-1} mancozeb (88 % purity, remaining composition not stated) and ^{3}H-thymidine for 18 h. UDS was assessed by autoradiography. Complete cytotoxicity was observed in cells at concentrations > 10 μg ml^{-1}. Cell survival at the remaining treatment concentrations (0.5 to 10 μg ml^{-1}) ranged from 97 to 37 %, compared to negative controls. No increase in the mean nuclear grain count per cell compared with concurrent negative controls was observed. The positive control substances used produced appropriate responses. Overall, results indicate that mancozeb did not induce DNA repair in rat hepatocytes in this assay (Unpublished, 1985f).

A published study is available in which the cytotoxic and DNA damaging effects of mancozeb in human blood peripheral lymphocytes (PBL) *in vitro* were assessed (Perocco *et al*, 1989). PBL cells were obtained from 2 healthy adult donors and were incubated in the presence of mitogen-stimulation for 52 h. For the last 4 h of incubation cells were also exposed to concentrations of up to 50 μg ml^{-1} mancozeb (98.6 % purity) in DMSO in the presence of ^{3}H-thymidine. Incubations were conducted both in the presence and absence of a S-9 mix microsomal metabolising system (no further details reported). In addition the number of viable cells was determined in unstimulated cells incubated with mancozeb in the absence of ^{3}H-thymidine for 4 h only, following which cells were stained with trypan blue. UDS was determined by assessment of ^{3}H-thymidine uptake by non-proliferating cells.

Toxicity in terms of both a reduction in ^{3}H-thymidine uptake and the number of viable cells (determined by the trypan blue staining technique) were observed in stimulated PBL cells in the absence of metabolic activation. In both instances the toxicity was dose-related, with decreases in the thymidine incorporation and the number of viable cells of up to 90 % at 50 μg ml^{-1}, compared with vehicle controls. No change in either ^{3}H-thymidine uptake or in cell viability was observed in cells in the presence of metabolic activation, compared with vehicle controls. UDS (data presented graphically only) in non-stimulated PBL was observed

only at > 5 μg ml^{-1} in the absence of metabolic activation. Thymidine incorporation was reduced by approximately 50 % compared with controls. Overall, the results of this assay indicate that at non-cytotoxic concentrations mancozeb did not induce DNA damage and therefore shows no direct-acting mutagenic potential in this system.

In a further study reported by the same Authors (Perocco *et al*, 1989) human PBL cells from 6 different donors were cultured with 0, 5, 15 or 25 μg ml^{-1} mancozeb (98.6 % purity) and incubated both in the presence and absence of metabolic activation for 72 h. The cells were then fixed and differential staining for sister chromatids performed. Metaphases with $\geq$ 44 chromosomes were scored for SCEs. Triplicate experiments were conducted. In 2 of 3 cultures, a 'slight' (13 - 20 %) increase in the number of sister chromatid exchanges (SCEs) was observed in the absence of metabolic activation and only at cytotoxic concentrations ($\geq$ 25 μg ml^{-1}). No effects were observed in the presence of metabolic activation at any concentration tested. Overall, since effects were only observed in 2/3 cultures and at only cytotoxic concentrations it can be concluded that mancozeb did not induce SCEs in this assay.

3.3.5.1.3.3 Ethylene thiourea

ETU is reported as giving negative results in chromosome aberration, SCE and UDS assays conducted *in vitro* in mammalian cells, rat liver cells or CHO cells. Positive results were reported in a mouse lymphoma assay for the induction of TFT-resistance in L5178/$TK^{+/-}$ cells (Unpublished, 1989b).

3.3.5.2 *IN VIVO* STUDIES

3.3.5.2.1 Studies in bone marrow cells

3.3.5.2.1.1 Zineb

A standard mouse bone marrow micronucleus test conducted to GLP standards is available. Zineb (91.5 % purity, other components not stated) was administered orally by gavage to groups of 15 CD mice per sex at a dose of 10,000 mg kg^{-1} as suspension in aqueous 1 % methylcellulose. Vehicle only and positive control groups were included. Groups of 5 male and 5 female zineb-treated and vehicle control animals were killed and bone marrow smears examined at 24, 48 and 72 h post dosing. Positive control smears were taken at 24 h only from 5 male and 5 female animals. The only outward signs of toxicity observed following zineb treatment were 'slight' pilo-erection and hunched posture for 6 h post dosing and abnormally coloured faeces between 21 and 48 h post-dosing. Zineb did not have a statistically significant effect on the number of micronucleated polychromatic (PCE) or normochromatic (NCE) erythrocytes compared with vehicle controls, or on the PCE/NCE ratio. Based on a consideration of the toxicokinetics of zineb it is presumed likely that the substance did reach the bone marrow. The positive control group displayed an appropriate response. This study indicates that orally administered zineb, at a sufficiently high dose (the maximum deliverable), did not exhibit mutagenic activity towards bone marrow erythroblasts in the mouse (Unpublished, 1985g).

In a poorly reported study 3 groups of rats (numbers per group not stated) were administered zineb (purity and composition not reported) at dose levels cited as 0, 1/15 or 1/200 the LD_{50}

(value not reported) for 6 d (Kiryushin, 1975). No indication of the actual dosage in mg kg^{-1} was presented. Some animals (numbers not given) were killed 24 h post-dosing and others at 10 d. Bone marrow smears of cells in metaphase were prepared from at least 6 animals and 100 metaphases per plate from each animal analysed for gap and structural rearrangements (chromatid and isochromatid breaks, translocations and aneuploid metaphases). A 'slight' increase (less than 2-fold) in the number of metaphase plates with breakages per 100 analysed, was observed for both dose levels at 24 h, with numbers returning to control levels at day 10. The Authors report that gaps and chromatid breaks constituted 90 % of the total damage recorded. No increase in the number of aneuploid metaphases was observed with either dose at either time point. This study indicates that orally administered zineb did not exhibit mutagenic activity towards bone marrow in the rats, although the limited reporting makes it difficult to drawn firm conclusions.

3.3.5.2.1.2 Mancozeb

In a well-conducted mouse bone marrow micronucleus test mancozeb (88 % purity, remaining composition not stated) was administered orally by gavage to groups of 15 Swiss-CD1 mice per sex, at a dose of 10,000 mg kg^{-1} as a suspension in aqueous 1 % methylcellulose. Vehicle only and positive control groups were included. Groups of 5 animals/sex from treated and vehicle control groups were killed and bone marrow smears examined at 24, 48 and 72 h post dosing. Positive control smears were taken at 24 h only. Clinical signs of toxicity observed following treatment were 'slight' pilo-erection, ptosis and hunched posture for 6 h post dosing. No increase in the number of micronucleated PCEs or NCEs and no statistically significant change in the PCE/NCE ratio were observed compared with vehicle controls. The positive control group displayed an appropriate response. This study indicates that orally administered mancozeb at high doses (the maximum deliverable), did not exhibit mutagenic activity towards bone marrow erythroblasts in the mouse (Unpublished, 1987b).

In a poorly reported study, a 'slight' but not statistically significant dose-related increase in the number of micronucleated PCEs (0.3 to 0.6 % in test samples compared with 0.1 % in concurrent negative controls) has been reported at 30 and 60 h post-dosing, in bone marrow smears taken from groups of 4 mice administered 250, 500 or 1000 mg kg^{-1} mancozeb (Dithane-M45™, 88 % purity, remaining composition not reported) via intraperitoneal injection (Henavathi and Rahiman, 1996a). No information regarding cytotoxicity or historical control values were reported, furthermore no information regarding changes in the PCE/NCE ratio was presented. Therefore due to the lack of necessary interpretative information no conclusions can be drawn from this study.

In a well-conducted *in vivo* rat bone marrow cytogenetics assay, concentrations of up to 5000 mg kg^{-1} mancozeb did not induce an increase in the number of chromosome aberrations following either single or repeat dosing. Groups of Fischer-344 rats were administered doses of 0, 500, 1720 or 5000 mg kg^{-1} mancozeb (88 % purity, remaining composition not reported) in corn oil, orally by gavage, either as a single dose (10 animals per dose) or once daily for 5 consecutive days (30 animals per dose). Positive and solvent control groups were also included in the study. Groups of 10 animals were killed at 6 (all acutely dosed animals), 24 or 48 h post dosing and bone marrow smears taken for cytogenetic analysis. One and 2 top dose animals, respectively, of the repeat or single dosed animals were found dead during the study. Clinical signs of toxicity observed were lethargy, piloerection in top and mid dose animals of both dosing regimens and to a lesser extent in low dose animals of the repeat dose

regimen. Positive control animals gave an appropriate increase in the incidence of chromosomal aberrations (Unpublished, 1984c).

In a second *in vivo* mammalian cell cytogenetic study, groups of 9 albino mice per dose were administered single intraperitoneal doses of 250, 500 or 1000 mg kg^{-1} mancozeb (Dithane-M45™, 88 % purity, remaining composition not reported) in 5 % DMSO (Henavathi and Rahiman, 1996b). Negative control animals were administered vehicle only. Three animals per group were killed 24, 48 and 72 h. One hundred well spread metaphases were assessed for the presence of chromatid and chromosome aberrations. Small dose-related increases in the number of metaphases with chromosome aberrations (without gaps) were observed (1 - 4.2 % compared with 1.3 - 1.6 % in negative controls). The maximal response for all three doses occurred at 48 h, with the frequency of aberrations declining towards control levels at 72 h. No concurrent or historical positive control data and no details regarding clinical signs of toxicity or cytotoxicity were reported. Therefore no conclusions from this study regarding the significance of this small increase can be drawn.

In a poorly and briefly reported *in vivo* mammalian cell cytogenetic study, groups of 4 male albino mice were administered single intraperitoneal doses of 0, 30, 40 or 300 mg kg^{-1} mancozeb (Dithane-M45™ 88 % purity, remaining composition not reported) in distilled water (Gautam and Kapoor, 1991). One animal from each dose group was killed on days 1, 2, 5 and 10 post-treatment and bone marrow smears were taken for analysis. Fifty metaphases were assessed for the presence of chromosome aberrations. A small dose-related increase in the number of metaphases with chromosome aberrations (without gaps) was observed (2 to 17 metaphases per 50 metaphases compared with a reported 0/50 in vehicle controls). There were no details regarding the time at which this control figure was derived or if there was any variation in the number of aberrations in controls over time. The maximal response for all three doses occurred at 2 days, with the frequency of aberrations declining towards control levels by day 10. No positive control animals were used in the study and no details regarding clinical signs of toxicity or cytotoxicity were reported. Due to the inadequate reporting of this study, the very low number of animals used, the low number of metaphases assessed and the lack of any positive control animal data no conclusions can be drawn regarding the significance of these small increases in the frequency in chromosomal aberrations.

3.3.5.2.1.3 Ethylene thiourea

Negative results are reported in micronuclei and SCE assays conducted *in vivo* in mice (Unpublished, 1989b).

3.3.5.2.2 Other *in vivo* studies

3.3.5.2.2.1 Zineb

The livers of groups of male Wistar rats and Swiss mice were assessed for DNA damage (reported as single strand breaks) and poly (ADP-ribose) polymerase (pADPRP) activity (as an indicator of enzymically mediated DNA repair), 4 h after being administered single doses of 0, 12.5, 25, 50 or 100 mg kg^{-1} zineb, in DMSO and vegetable oil, via intraperitoneal injection (Scarabelli *et al*, 1993). In rats endogenous liver pADPRP activity was found to be increased 4.5 fold over that of controls in animals given 50 or 100 mg kg^{-1} of zineb. DNA

damage was also found to be increased in rat liver samples but in this instance only in the dose groups receiving 12.5 and 50 mg kg^{-1} zineb (3.2 and 2.6 fold, respectively). No change was observed at the top dose tested. In contrast no effect in the pADPRP activity or in the incidence of DNA damage was observed in any of the mouse liver samples. The toxicological significance of these findings is unknown.

3.3.5.2.2.2 Ethylene thiourea

Negative results are reported in dominant lethal mutation and sperm abnormality assays in rodents (Unpublished, 1989b; IPCS 1988).

3.3.5.2.3 Human data

3.3.5.2.3.1 Zineb

A briefly reported study investigating possible clastogenic effects in zineb-exposed workers is available (Plinskaya, 1974). Blood samples were taken from 15 workers (3 males and 12 females) employed as fitters, packers and drivers in a plant manufacturing zineb. The average age of the workers was 27 years and they had been employed in the plant for between 1 and 10 months. Control blood samples were taken from 5 healthy volunteers (1 male and 4 females) of a similar age. Test and control subjects were selected on the basis of a questionnaire aimed at eliminating persons exposed to other clastogenic confounders. No details of which confounders were used for the selection process were reported. No information regarding the underlying health of the sample test population was reported.

The Authors indicate that the test workers were exposed only to zineb dust. No details regarding exposure concentrations, particle size, possible contaminants or duration of exposures are presented. It is however reported that the respirators worn by the workers were inadequate and that 0.15 mg of zineb was measured in the washings of nasal mucosa taken form workers post-shift. It is not clear if this level is a peak or an average measurement, if it relates to one or all types of working environment or from how many workers the samples were taken. No details regarding the measurement protocol are presented. Cultures of peripheral blood lymphocytes from test and control subjects were incubated for 52 to 57 h (no further protocol details were reported). Chromosome preparations were obtained and 200 metaphases per culture studied for aberrations.

An increase in the percentage number of metaphases with aberrations (including gaps) in the workers of 5 to 8.5 % (mean 6 %) for both males and females, across all 3 job types and for all durations of employment, compared with 1 % in controls were reported. There was no increase in the number of aberrations per aberrant metaphase, in workers compared with controls. Due to the limitations in the study (i.e. low sample numbers) and in its reporting, no conclusions regarding the quality of the study, the reliability or toxicological significance of the results can be drawn.

3.3.5.2.3.2 Mancozeb

The incidence of chromosomal aberrations and sister-chromatid exchanges have been analysed in short-term cultures of peripheral human lymphocytes of 44 workers occupationally exposed for up to 2 years to mancozeb during the production of the pesticide Novozir Mn80 (80-85 % mancozeb in final product) and in 30 control (non-exposed) persons

(Jablonická *et al*, 1989). The dust concentration of the final product in air, in the packing area, was reported as 3.25 to 7.55 mg m^{-3}, no details regarding method or duration of measurement were reported. Levels of dust (0.99-4.89 mg m^{-3}), ammonium (0.66-54.70 mg m^{-3}) and carbon disulphide (8.50-120.90 mg m^{-3}) in the centres of the working zones were also reported (no further details were reported). The average age of the exposed persons tested was 28 years for men and 31 years for women. The controls were employed in the same plant but were said to have had no occupational contact with any chemicals. The controls were reportedly matched to the exposed for habits (no further details reported), social positions and age, but not for smoking. In the exposed group there were 25 smokers and 19 non-smokers, whilst in the control group there were 5 smokers and 25 non-smokers. Objective physical and medical examinations and laboratory tests did not indicate any adverse effects in the exposed workers. No individual data were reported. The mean group data show a slight increase in the number of aberrations (excluding gaps) in the exposed workers (2.7 %) compared with controls (1.3 %). However as the number of control individuals is lower than the number of exposed individuals and there is no adjustment for smoking, the toxicological and statistical significance of these findings are unclear. The data indicate that in this study, there is no statistically significant change in the frequency of SCEs between exposed and non-exposed populations (9.2 ± 1.9 SCEs/cell in exposed group compared with 7.8 ± 1.0 in non-exposed controls). It is important to note that not all of the study individuals were assessed for SCEs (24/44 exposed individuals and 27/30 non-exposed individuals), therefore it is difficult to assess whether bias might have occurred. Due to study and reporting limitations no conclusions can be drawn from this study.

Cytogenetic analysis (SCE and chromosomal aberrations) was performed using the blood of seasonal workers exposed to EBDCs (for experimental details see Section 3.3.4.5) during crop spraying (Steenland *et al*, 1997)**.** Cytogenetic analysis was conducted on blood samples from; 31/49 sprayers, 13/14 landowners and 30/31 unexposed controls. The average age and percentage of smokers in the test populations were; 26 years and 70 % smokers for sprayers, 32 years and 50 % smokers for landowners and 22 years and 67 % smokers for unexposed controls.

With regard the cytogenetic analysis (SCE) both unadjusted and adjusted (for age and smoking) data indicate that the sprayers (8.50 ± 0.22 and 8.52 ± 0.19 respectively) had a slightly but statistically significantly higher number of SCE per cell compared with non-exposed controls (7.80 ± 0.18 and 7.90 ± 0.2 respectively). Values for landowners were comparable with non-exposed controls. The total numbers of chromosome aberrations (reported as translocations) determined from both unadjusted and adjusted data (for age and smoking), were statistically elevated in both sprayers (4.61 ± 0.94 and 6.07 ± 0.97 respectively and landowners (6.00 ± 1.00 and 5.44 ± 1.00 respectively) compared with non-exposed controls (2.76 ± 0.54 and 4.59 ± 0.82 respectively). In each test population over 50 % of the aberrations were reciprocal translocations (not dicentrics, insertions, rings or fragments). It is not clear from the report if gaps were observed or scored.

The significance of EBDC exposure with respect to the development of these marginal cytogenetic changes is uncertain as there is a potential for the confounding exposure to other genotoxic pesticides (captan). Overall, this study does not provide any useful information regarding possible cytogenetic effects of occupational exposure to EBDCs.

3.3.5.2.3.3 Ethylene thiourea

No data were submitted.

3.3.5.3 SUMMARY OF GENOTOXICITY

Cytogenetic analysis of the peripheral lymphocytes of workers potentially exposed to zineb and/or mancozeb have been carried out. However, due to limitations in the design and reporting of the studies no firm conclusions could be drawn from them. Zineb has been tested for its mutagenic potential in a variety of *in vitro* and *in vivo* assays and in rodent somatic cell *in vivo* assays. The balance of data available clearly indicates a lack of genotoxic activity for zineb *in vitro* in either bacteria or mammalian somatic cells and *in vivo* in somatic cells following oral dosing. This profile of a lack of mutagenic potential is supported by the negative results in equivalent studies for both the structurally related mancozeb and the common metabolite ETU. No studies in germ cells are available. However, given the lack of genotoxic activity in somatic cells both *in vitro* and *in vivo* there is no basis for any concern that zineb could be genotoxic to the germ cells.

3.3.6 CARCINOGENICITY

3.3.6.1 *IN VITRO* STUDIES

A published *in vitro* BALB/C 3T3 cell transformation study is available (Perocco *et al*, 1995). Cytotoxicity was assessed by incubating cells with concentrations up to1 μg ml^{-1} zineb (78.6 % purity) in DMSO, both in the presence of phenobarbital-induced rat liver S9 for 4 h and in the absence of S9 and for 72 h. Cells were maintained in culture for a further 7 - 10 d. Cytotoxicity was observed in the absence of S9 at ≥ 0.5g ml^{-1} (40 % at 0.5g ml^{-1} to zero at 1 μg ml^{-1}), compared with negative controls. No evidence of cytotoxicity was observed at 0.1 μg ml^{-1} in the absence of S9 or at any concentrations in the presence of S9.

No transforming effects were detectable in treated cultures in either the presence or absence of S9. The positive control substances used produced appropriate responses. Results indicate that zineb does not transform BALB/c 3T3 cells.

3.3.6.2 ORAL

3.3.6.2.1 Rat

3.3.6.2.1.1 Zineb

Groups of 10 male and 10 female albino rats (strain not stated) were fed diets containing 0, 500, 1000, 2500, 5000 or 10000 ppm zineb (purity and composition not reported; described as a '65 % commercial wettable powder') for 2 years (Blackwell-Smith, *et al*, 1953). The Authors calculated the mean doses of zineb received as equivalent to 0, 30, 60, 150 or 600 mg kg^{-1} d^{-1}, respectively. The animals were weighed weekly. Blood samples were taken at 11 and 22 months for analysis of erythrocyte count, haemoglobin concentration and differential white cell count. Gross and microscopic examinations were conducted on all survivors at 22 months and on all decedents (except those in which autolysis was advanced). The tissues examined included heart, lung, thyroid, liver, stomach, spleen, kidney, small and large intestine and the gonads.

At 52 weeks 15/120 animals had died, the mortality profile per dose group being respectively; 1,1,0,2,4,2 in males and 1,1,0,1,0,2 in females. At 104 weeks 74/120 treated animals had died, the profile of mortalities per dose group was respectively; 7,7,5,8,7,7 for males and 4,6,2,5,7,9 for females. This low survival rate in treated animals makes the study data generated (i.e. histopathology) very difficult to interpret. Three control animals had died during the study. At study termination an overall decrease in average body weight of approximately 15 % was observed in top dose females had died compared with controls. No treatment-related change in the average male body weight gain was observed during the study period. No information regarding clinical signs of toxicity was reported. No treatment-related changes in haematology were observed amongst the limited parameters recorded.

At necropsy, evidence of thyroid hyperplasia was observed in both control and treated animals, with the severity of response being greater in treated animals compared with controls. Thyroid hyperplasia was graded from 0 to 5. Eight from 17 surviving control animals presented thyroid hyperplasia of grade 2 or less, no effect was observed in the remaining 9 animals. Thyroid hyperplasia of grade 3 and above was observed in all surviving treated animals. Treated animals also presented a dose-related increase in terms of both the severity of thyroid hyperplasia and incidence of response. The Authors report that complications in interpreting the thyroid to body weight ratios (the small number of survivors, obesity and disease [no details given]) meant that no conclusions regarding this endpoint could be drawn.

Kidney congestion, nephritis and nephrosis were also observed in 6/19 top dosed animals, males and females. One animal in each of the 1000, 2500 and 5000 ppm dose groups showed evidence of nephritis. No kidney effects were observed in any animal at 500 ppm. No effects in any other tissues or organs examined were reported.

Overall, based on the low number of animals tested and the high mortality rate no meaningful conclusions can be drawn from this study with respect to carcinogenicity.

A published report of a limited study in rats is available, in which one group of 60 random-bred rats (strain not reported, referenced only as of ‘common stock’) was administered 285 mg kg^{-1} d^{-1} zineb (90 % purity, remaining composition not reported) in water, by oral gavage for 22 months (Andrianova and Alekseev, 1970). A control group of 48 animals receiving vehicle only was also used. Animals were weighed monthly. At termination tissues and organs suspected of harbouring tumours were examined histopathologically. No details of the exact tissues or organs analysed are presented. No information regarding the conduct of any observations for clinical signs of toxicity, haematological or biochemical changes are reported.

Fifty treated animals and 2 control animals died before termination of the study. No information regarding the likely causes of death are reported. No details of the measured body weights were reported. In the surviving 10 treated animals 2 were found to have tumours, 1 had an adenocarcinoma of the large intestine and the other had an intestinal lymphosarcoma. One control animal was reported to have a fibrosarcoma (location not reported). Due to limitations of the study (the lack of clinical, haematological or biochemical observations), the high mortality rate observed in treated animals, the differing types of tumours observed and the general poor reporting of the study, no firm conclusions regarding potential tumourogenicity can be drawn from this study.

Overall, based on the data available no conclusions regarding the carcinogenic potential of zineb in rats following oral administration can be made.

3.3.6.2.1.2 Mancozeb

In a study conducted to GLP standards, groups of 70 Sprague-Dawley rats of each sex received mancozeb (88 % purity, remaining composition not reported) continuously in the diet at dietary concentrations of 0, 25, 100 or 400 ppm. Ten animals per sex per group were killed after 52 weeks. The remaining 60 animals per sex per group were dosed for the full 104 weeks of the study. The mean doses of mancozeb received, as a function of body weight, were calculated from food consumption measurements as 0, 1, 4 or 16 mg kg^{-1} d^{-1} in males and 0, 1, 5 or 21 mg kg^{-1} d^{-1} in females (Unpublished, 1992d).

The animals were monitored throughout for clinical signs of toxicity, body weight and food consumption. Blood samples were taken from 10 animals per sex per group for extensive haematological and biochemical analysis (including T_4 measurements) at 26, 52, 78 and 104 weeks. Urine samples were taken from the remaining 50 animals per sex per group for extensive analysis at 26, 52, 78 and 104 weeks. Prior to termination animals underwent ophthalmoscopic examinations. At necropsy, all standard organs were weighed, macroscopic observations on a wide range of tissue and organs recorded and extensive histopathological examination performed.

At 104 weeks there was no evidence of impaired survival due to mancozeb; the mortality profile per dose group was respectively; 28, 26, 28 and 27 in males and 28, 34, 30 and 34 in females. No clinical signs of toxicity were observed in any of the animals. Relative to controls, body weight gain was unchanged in males and females at 25 or 100 ppm. Overall body weight gain at 400 ppm in both males and females was reduced by just 5 to 6 %. Food consumption was similar across all groups.

Haematological analysis revealed no treatment-related changes, in any of the animals examined. Clinical chemistry measurements revealed a slight statistically significant reduction in thyroxine (T_4) levels (15 to 20 %) in males and females at 400 ppm at 26 and 52 weeks. Levels were comparable with those in control animals at 78 and 104 weeks. No other changes in clinical chemistry were revealed. Urinalysis and ophthalmoscopic examination did not reveal any treatment-related changes in any of the groups.

At both the 52 and 104 week necropsies absolute and relative organ weights of treated animals were comparable with controls. The only observations of adverse effect in treated animals compared with controls at necropsy at 104 weeks were; thyroid masses in 2/50 and 6/50 at 100 and 400 ppm respectively, and an increase in the incidence of unilaterally blue discoloured testes (11/50 compared with 3/50 in controls) and unilaterally small testes (10/50 compared with 1/50 in controls) in decedent and surviving male at 400 ppm. No other abnormal pathological findings were observed.

No microscopic abnormalities were seen in any of the animals examined at the 52 week interim sacrifice. At the 104-week examination changes were confined to the thyroid, and consisted of ‘slight’ hyperplasia in all animals and an increase in raised follicular epithelium in males (9/50 versus 5/50 in controls) and females (10/50 versus 2/50 in controls) at 400 ppm. No microscopic effects in the testes, spermatogenesis or seminiferous tubules were observed. No other non-neoplastic effects were observed. No treatment-related neoplastic changes were observed in any groups.

Overall, these data indicate that the thyroid is a target organ for mancozeb toxicity and that mancozeb was not tumourigenic in rats following exposure via the oral route under the conditions of this study. The observed testicular effects are believed to be of no toxicological significance, as no histopathological evidence of an effect in the testes was observed and the discolouration was also observed in control animals. A NOAEL for systemic toxicity of 100 ppm (4-5 mg kg^{-1} d^{-1}) can be determined from this study.

In a combined chronic toxicity/ oncogenicity study in CD rats, conducted to GLP standards, 62 animals per sex per group received mancozeb (84 % purity, containing approximately 1.1 % ETU) in the diet at concentrations of 0, 20, 60, 125, or 750 ppm for up to 2 years. An additional interim sacrifice group of 10 rats per sex per group received these doses for 12 months only. The mean intake of mancozeb, rounded to the nearest whole numbers, was 0, 1, 2, 5 or 31 mg kg^{-1} for males and 0, 1, 3, 7 or 40 mg kg^{-1} for females (Unpublished, 1991a).

Throughout the study the animals were observed for clinical signs of toxicity and body weight and food consumption was monitored. Blood and urine samples were taken at 3, 6, 12,18 and 24 months for haematology, clinical chemistry (including T_4 and TSH) and urinalysis. Animals were also examined for ophthalmic changes at 24 months. At sacrifice after 12 and 24 months animals were subjected to macroscopic examination, weighing of organs (adrenals, brain, heart, kidneys, liver, testes and thyroids) and extensive histopathological investigation.

At study termination (24 months) the mortality profile per dose group was; 26, 29, 39, 16 and 21 % in males and 37, 53, 39, 39 and 42 % in females, with no evidence that mancozeb administration specifically increased mortality. The only clinical sign of toxicity observed was diarrhoea in males at 750 ppm. No significant treatment-related changes in body weight gain or in food consumption were observed. No treatment-related ophthalmic changes were observed in any test group.

Investigations revealed no treatment-related changes in any dose group in haematology, clinical chemistry or urinalysis compared with controls, at any time point. At 750 ppm thyroid function analysis revealed: a statistically significant reduction in T_4 levels in males at 24 months (16 %) and in females at 18 and 24 months (22 and 21 %), compared with controls; a statistically significant increase in TSH levels in males at 12 and 18 months (33 and 52 % respectively), and in females at 6 months only (76 %), compared with controls. No effects on T_4 or TSH levels were measured in animals up to and including 125 ppm mancozeb.

At post-mortem there were no statistically significant changes in organ weight that could be attributed to mancozeb at either 12 or 24 months. At 12-month necropsy no macroscopic findings were reported in males or females at any exposure concentrations. At 24 months no abnormal gross pathological findings were observed in males or females at concentrations up to 125 ppm. Necropsy of animals at 750 ppm revealed an increased incidence of enlarged thyroids in males (14/61 against 2/60 in controls) and females (5/61 against 0/62 in controls) and of thyroid masses in males only (9/61 against 0/60 in controls). No findings were reported in animals at concentrations up to 125 ppm.

Histopathological examination of animals at concentrations up to and including 125 ppm revealed no evidence of any effects (non-neoplastic or neoplastic) attributable to mancozeb at

either 12 or 24 months. The most common non-neoplastic finding observed at 12 and 24 months in both males and females at 750 ppm was thyroid follicular cell hypertrophy/hyperplasia (10/11 males and 10/11 females compared with 0/12 in control animals at 12 months, and 34/61 males and 24/61 females compared with 1//60 and 1/62 in control animals at 24 months). In addition at 24 months thyroid follicular cell nodular hyperplasia (13/61 in males and 11/61 in females compared with 0 in control animals) was observed.

The most common neoplastic findings observed in both males and females at 750 ppm were thyroid follicular cell adenomas (20/61 in males and 6/61 in females, compared with 0 in controls) and thyroid follicular cell carcinomas (14/61 in males and 4/61 in females, compared with 0 in controls). There were no other findings that could be directly attributed to mancozeb treatment.

Overall, the results of this study indicate that the target organ for mancozeb is the thyroid gland, and that a 24 months oral dietary administration of mancozeb at concentrations of 750 ppm for 24 months induces thyroid tumours in rats. There were no toxicologically significant effects of mancozeb administration up to and including 125 ppm in the diet, equivalent to 5 mg kg^{-1} for males and 7 mg kg^{-1} for female rats respectively. At 750 ppm (equivalent to 31 mg kg^{-1} for males and 40 mg kg^{-1} for females respectively), there was evidence of reduced serum T_4 levels, increased serum TSH levels, thyroid enlargement, hyperplasia and hypertrophy of thyroid follicular cells. NOAELs for systemic toxicity and carcinogenicity of 125 ppm (5-7 mg kg^{-1} d^{-1}) can be determined from this study.

3.3.6.2.1.3 Ethylene Thiourea

In a chronic toxicity study in Sprague-Dawley rats, reportedly conducted to GLP standards, 20 animals per sex per group received ETU (96 % purity) continuously in the diet at concentrations of 0, 0.5, 2.5, 5.0 or 125 ppm, for up to 2 years. An additional interim-sacrifice group of 10 rats per sex per group received these doses for 12 months only. The mean intake of ETU was 0, 0.04, 0.17, 0.37 or 8.91 mg kg^{-1} for males and 0, 0.05, 0.25, 0.49 or 13.57 mg kg^{-1} for females. Throughout the study the animals were observed for clinical signs of toxicity, and body weight and food consumption was monitored. Blood and urine samples were taken from 10 animals per sex per group at weeks 25, 78 and 104 in the main study animals and at week 51 from a satellite group of animals. Additional blood samples were taken at weeks 24, 75 and 102 in the main study animals and at week 50 from a satellite group of animals for thyroid function analysis (T_3, T_4 and TSH). Animals were also examined for ophthalmic changes at 103 weeks. At sacrifice after 12 and 24 months animals were subjected to comprehensive macroscopic examination, weighing of organs and extensive histopathological investigation (Unpublished, 1992c).

Survival to study termination was moderate, with 29 to 43 % survival in treated males compared to 57 % in controls, and 29 to 51 % survival of treated females compared with 46 % in controls, with no evidence that ETU administration specifically increased mortality. No clinical signs of toxicity were observed in any groups throughout the study. No significant changes in body weight gain or in food consumption were observed across all groups. No treatment-related ophthalmic changes were observed in any test group.

Investigations revealed no treatment-related changes in any dose group in haematology or urinalysis compared with controls, at any time point. No biochemical effects were observed

in males up to 2.5 ppm and in females up to 5.0 ppm. Statistically significant changes in biochemistry observed, compared with controls included; increased G-GT in males at 125 ppm at weeks 51 and 78 (158 and 62 %, respectively), increased bilirubin levels in males at 125 ppm at weeks 51 and 78 (19 and 20 %, respectively), decreased urea levels in males at 125 ppm at week 78 only (23 %), and increased uric acid levels in females at 125 ppm at weeks 25 and 104 (55 and 24 %, respectively).

Thyroid function analysis revealed a statistically significant reduction in T_4 levels in both males and females at 125 ppm, at week 29 (42 and 45 %, respectively) and week 50 (36 % in both), compared with controls. T_3 levels were statistically significantly increased in males at 5 and 125 ppm at week 29 (22 and 53 %, respectively), in both males and females at 125 ppm at week 50 (34 and 21 %, respectively), and in males at 125 ppm at week 78 (31 %) compared with controls. TSH levels were statistically significantly increased in males and females at 125 ppm at week 29 (177 and 94 %, respectively), week 50 (94 and 37 %, respectively), at week 75 (by 94 and 57 % respectively), and in males only at week 102 (66 %), compared with controls.

At interim and terminal sacrifice no effects on organ weights were found in any organs in animals at concentrations of ETU up to 5 ppm. The only statistically significant change in organ weight that could be attributed to ETU was increased absolute thyroid weights in males (28 % at week 52 and 33 % at week 104) and females (16 % at week 52 and 43 % at week 104) at 125 ppm.

At interim necropsy no macroscopic findings were reported in males or females at any exposure concentrations. At terminal necropsy no gross pathological findings were observed in males at concentrations up to 5 ppm or females at concentrations up to 125 ppm. Necropsy of males at 125 ppm revealed an increased incidence of enlarged thyroid glands (6/20 compared with 1/20 in controls).

Histopathological examination of animals at concentrations up to 5 ppm revealed no evidence of any effects (non-neoplastic or neoplastic) attributable to ETU at either 52 or 104 weeks.

The most common non-neoplastic findings observed at 52 weeks at 125 ppm were; minimal to moderate diffuse thyroid follicular cell hyperplasia in males and females (10/10 males and 10/10 in females compared with 6/10 and 2/10, respectively in control animals), nodular thyroid hyperplasia and hypertrophic pituitary foci in males only (6/10 and 7/10 respectively, compared with 0/10 and 1/10 in control animals). There were no other observed findings that could be directly attributed to ETU administration.

At 104 weeks the most commonly observed findings at 125 ppm were; diffuse thyroid follicular cell hyperplasia in both males and females (10/19 males and 17/20 females compared with 0/18 and 1/19 in control animals, respectively) thyroid follicular cell nodular hyperplasia in males only (7/19 compared with 0/18 in control animals), depletion of zymogen granules in pancreas in males only (8/19 males compared with 1/18 in control animals) and pigment deposition in the pancreas in males only (10/19 males compared with 5/18 in control animals). There were no other observed findings that could be directly attributed to ETU administration.

At 52 weeks the only neoplastic finding observed was thyroid follicular cell adenoma (3/10 compared with 0 in controls) in males only at 125 ppm. At 104 weeks the only treatment-

related neoplastic findings were observed in males only at 125 ppm and comprised thyroid follicular cell adenoma (3/19 compared with 1 in controls), thyroid follicular cell adenomas (4/19 compared with 1 in controls) and follicular cell carcinoma (2/19 compared with 0 in controls).

Overall, the results of this study indicate that ETU induced thyroid tumours in rats following 52 week and 104 weeks oral administration at concentrations of 125 ppm. There were no toxicologically significant effects of ETU administration up to 5 ppm in the diet, equivalent to 0.37 mg kg^{-1} for males and 0.49 mg kg^{-1} for female rats respectively.

A complex NTP study designed to assess the carcinogenic effects of ETU administered perinatally (via dams diet), to adults only, and combined prenatal and adult exposure, for 2 years in F/344 rats is available. Groups of 50 females received ETU (99 % purity) continuously in the diet at concentrations of 0, 9, 30 or 90 ppm for 2 weeks prior to mating, throughout gestation and lactation (see Section 3.3.7.1.3). Dams were mated with unexposed males. On day 4 postpartum litters were reduced to a maximum of 8 pups. Post-weaning (day 28 postpartum) pups were separated by sex within maternal dose groups, (including the controls), and at week 8 animals were grouped, 60 animals per sex per group, and exposed to ETU concentrations of 0, 25, 83 or 250 ppm for up to 2 years (dose selection study, see Section 3.3.4.1.1.3). The final dosing regimens for the respective groups over 2 years were; 0:0 for overall control animals (these animals received no ETU exposure throughout life), 90:0 for perinatally only exposed animals (exposed during gestation and lactation to weaning), 0:83 or 0:250 for adult only exposed animals (these animals were not exposed *in utero* or during lactation), and 9:25, 30:83, 90:83 and 90:250 ppm for combined perinatal and adult exposed animals (these animals received ETU throughout life). The mean intake of ETU could not be calculated, as food consumption data were not presented. An additional interim-sacrifice group of 10 rats per sex per group received these doses for 9 months only (Unpublished, 1989b).

Throughout the study the animals were observed for clinical signs of toxicity, and body weight and food consumption was monitored. At interim sacrifice blood and urine samples were taken for haematological, clinical chemistry and urinalysis. At both interim and terminal sacrifice blood samples were taken for thyroid function analysis (T_3, T_4 and TSH levels). All animals at interim sacrifice, and decedents and survivors of the full study were necropsied and standard organ weights recorded. Extensive histopathological assessment was conducted on all animals.

All animals survived to the interim sacrifice. Survival rates at study termination were comparable to that of the 0:0 overall control group, for all animals across the different exposure regimens, except for the 90:250 ppm exposed males, whose survival rate was significantly decreased, by approximately 50 % compared with the 0:0 overall control group. No clinical signs of toxicity were observed in any groups throughout the study. No significant changes in body weight gain or in food consumption were reported across all groups at interim sacrifice. At terminal sacrifice the mean body weight of males in the 90:250 ppm exposure group was reduced by 18 % compared with 0:0 control animals No other treatment-related changes in body weight were reported in any other animals across the exposure regimens.

At interim sacrifice organ weights of all exposed animals were comparable with controls. In males absolute liver weights were marginally, but statistically significantly increased in 0:83,

0:250 and 90:250 ppm exposure groups, by 11, 16 and 19 %, respectively, compared with 0:0 controls. Thyroid weights of males and females of the 0:250 and 90:250 were reportedly increased, but not statistically significantly (no numerical details presented).

No information regarding organ weight changes at study termination was reported.

Investigations revealed no treatment-related changes in any dose group in haematology, clinical chemistry or urinalysis compared with controls.

Thyroid function analysis of interim killed animals revealed a statistically significant and dose-related reduction in T_4 levels in males (18 to 46 %) and females (27 to 52 %) in all exposure groups, except in animals in the 90:0 group in which levels were comparable to 0:0 controls. T_4 levels in males at terminal sacrifice were of similar magnitude to the interim levels, but in fewer exposure groups, with no significant changes in the 90:0, 9:25 ppm groups. In females of the 90:250 ppm group only had significantly reduced T_4 levels (41 %), with levels in the remaining exposure groups comparable with 0:0 controls.

At interim sacrifice, TSH levels in males were increased in most exposure groups, by between 46 and 61 %, except in the 0:83, 90:0 and 9:25 ppm exposure groups in which the increase was in the range 5 to 24 %, compared with 0:0 controls. In females, TSH levels were increased, in all but the 90:0 exposure group, in a dose-related manner by between 49 and 145 %, compared with 0:0 controls. Levels in the 90:0 group were comparable with controls.

At terminal analysis TSH levels in males and females of the 90:83, 90:250 and 30:83 ppm groups were between 1 and 5 fold greater than those in the 0:0 controls. Levels in animals of the 0:83 and 9:25 (males only) ppm groups were increased by approximately 50 %, compared with controls, and levels in the remaining groups were comparable with controls.

At both interim and terminal sacrifice T_3 levels in both males and females were variable being both elevated and decreased compared with controls, with no identifiable trend or dose-response relationship being observable.

Histopathological examinations of interim and terminally killed animals revealed an increased incidence of thyroid follicular cell hyperplasia across all exposure regimens. In the interim killed animals the incidence of effect was 8 to 10 males and 5 to 10 females of the 0:83, 0:250, 90:83, 30:83 and 90:250 ppm exposure groups. Incidences were lower in the remaining exposure groups (0 to 4 animals), compared with the 0:0 control group (0 animals). At termination the incidences in males and females ranged between 20 and 94 %, with the lower incidence being in the 90:0 exposure group, compared with 8 and 0 % in the respective 0:0 control groups.

Follicular cell adenomas were observed in 3 males and 1 female of the 90:250 ppm exposure group at interim sacrifice. No adenomas were observed in any other animals across the exposure groups at the sacrifice. At terminal sacrifice, follicular cell adenomas were observed in males and females in the majority of exposure groups, the incidence of tumours ranging from 16 to 68 %, compared with 0 and 2 % in male and female 0:0 control animals. The lower incidences (10 to 21 %) for each sex were observed in the 0:83, 30:83 and 90:83 ppm exposure groups. No significant increase in adenomas was observed in animals of the 90:0 exposure group compared with 0:0 controls.

No evidence of follicular cell carcinoma was reported at interim sacrifice. At terminal sacrifice, follicular cell carcinomas were observed in males and females, the most significant increases in incidence being observed in the 0:250 and 90:250 ppm exposure groups (52 and 88 % in males and 16 to 34 % in females, respectively, compared with 2 and 4 % in 0:0 controls). Incidences in males and females in other exposure groups were slightly increased, between 2 and 12 %, compared with 0:0 controls.

No other neoplastic or non-neoplastic effects which could be directly related to ETU exposure were observed in any animals exposed as adult only (0:83 or 0:250 ppm) or perinatally only (90:0 ppm) or to combined perinatal and adult exposures of ETU (9:25, 30:83, 90:83 or 90:250 ppm), compared with 0:0 controls.

Five of the 50 males and 4/50 females receiving 90:250 ppm, and 2/50 males and 3/50 females at 90:83 ppm presented with Zymbal's gland carcinomas, compared with control incidences of 3/50 or 2/50 at 0:250 ppm, respectively, or 3/50 and 0/50 at 0:83 ppm, respectively, or 1/50 at 0:0 ppm (both sexes). The NTP-historical incidence of this tumour type in rats in 2-year studies is reported as 1 % in males and females. Mononuclear cell leukaemia was observed in 29-35/50 males compared with 22-32/50 in controls, and in 19-29/50 females compared with 18-27/50 in controls. Slight but not dose-related increases in the incidences renal tubular cell hyperplasia (0-7/50), adenomas (1-4/50) and carcinomas (0-1/50) were observed in male rats across the exposure groups, including controls. Considering the high spontaneous control incidences of these tumours, the increases in the incidences of these tumour types as observed in this study are not considered to be a direct consequence of ETU exposure.

Overall, the results of this study indicate that ETU administered orally either perinatally, to adults only or in combination, at respective concentrations of 90:0, 83:0 or 9:25 ppm and above, induced a significant increase in the incidence of follicular cell hyperplasia and TSH levels, and a reduction in T_4 levels compared with untreated controls. Perinatal exposure alone had no effect on the incidence of thyroid neoplasms. Adult only exposure to 250 ppm ETU led to an increase in the incidence of thyroid neoplasms. However combined perinatal and adult exposure to ETU (90:250 ppm) resulted in significantly increased incidences of follicular cell neoplasms, compared with adult only exposures.

3.3.6.2.2 Mouse

3.3.6.2.2.1 Zineb

A published paper that briefly summarises the results from the testing of more than 100 different substances for carcinogenicity in mice, as part of the USA National Cancer Institute Testing Programme in the 1960's is available (Innes *et al*, 1969). Very little information is given for each individual study. Eighteen mice per sex (aged 7 d), from each of two different strains (C57BL/6 AKR and C3H hybrids), received zineb (97 % purity) at 464 mg kg^{-1} in 0.5 % aqueous gelatin by oral gavage on days 1 - 21 followed by 1300 mg kg^{-1} in the diet (equivalent to 166 mg kg^{-1} d^{-1}) from day 21 until termination at 18 months. The doses given were stated to be the maximum tolerated dose for infant mice, but not necessarily for adults (no further details given). At study termination 15, 18, 16 and 16 animals in each of the four test groups, respectively had survived. Negative and positive control groups were also included in the study. The Authors report that no increase in tumour production caused by

zineb was observed. Due to the low number of animals tested and the limited study detail reported no conclusions can be drawn from this study.

In a poorly reported study oral doses of either 3500 mg kg^{-1} zineb (purity and composition not reported) in 1 % starch solution were administered to strain A and C57BL mice once weekly for 6 weeks (Chernov and Khitsenko, 1969). Untreated and positive (urethane) control animals were also used. The number of animals per group at the start of the study was not reported. Animals were killed 3 months after study onset and assessed for any tumourigenic effects. A 'slight' but not statistically significant increase in the incidence of lung adenomas was observed in C57BL mice 6/79 (8 %) mice compared with 0/87 in untreated controls and 18 % in positive controls, and in strain A mice 35/101 (35 %), compared with 30/97 (31 %) in untreated controls and 100 % in positive controls. No historical control data were reported. No observations of any other tumour types were reported. No conclusions with respect to carcinogenicity can be drawn from this limited study.

In addition groups of C57BL mice were administered 1750 mg kg^{-1} zineb (purity and composition not reported) via oral gavage once weekly for 11 weeks (Chernov and Khitsenko, 1969). Untreated and positive (urethane) control animals were also used. The number of animals per group at the start of the study was not reported. Animals were killed periodically between 2 weeks and 6 months after study onset and assessed for any tumourigenic effects. A 'very slight' but not significant increase in the incidence of lung adenomas was observed in 2/29 animals (7 %) compared with 0/59 in controls. No historical control data were reported. No other tumour types were identified. Due to the low number of animals surviving and the poor reporting of this study, no conclusions can be drawn from it.

Overall, based on the data available no conclusions regarding the carcinogenic potential of zineb in mice following oral administration can be made.

3.3.6.2.2.2 Mancozeb

In a carcinogenicity study in CD-1 mice, conducted to GLP standards, 70 animals per sex per group received mancozeb (83 % purity, remaining composition not reported) in the diet at concentrations of 0, 30, 100 or 1000 ppm for up to 78 weeks. An additional interim-sacrifice group of 24 mice per sex per group received these doses for 52 weeks only. The mean intake of mancozeb, expressed as a function of food consumption and body weight and rounded to the nearest whole numbers, was 0, 4, 13 or 131 mg kg^{-1} for males and 0, 5, 18 or 180 mg kg^{-1} for females (Unpublished, 1991b).

Throughout the study the animals were observed for clinical signs of toxicity, and body weight and food consumption was monitored. Blood samples were taken from 15 animals per sex per group at 52 and 78 weeks for haematological analysis and thyroid function assays (T_3, T_4 and TSH levels). No additional clinical chemistry or urinalysis was conducted. At sacrifice after 52 or 78 weeks animals were subjected to macroscopic examination, weighing of organs (adrenals, brain, heart, kidneys, liver, testes and thyroids) and extensive histopathological investigation.

At study termination (78 weeks) the mortality profile per dose group was respectively; 21, 22, 9, and 17 % in males and 21, 17, 14 and 19 % in females, with no evidence that mancozeb

administration specifically increased mortality. No clinical signs of toxicity were observed in any of the animals. Relative to controls, body weight gain was unchanged in males and females at 30 or 100 ppm. In both males and females at 1000 ppm body weight gain was reduced by 14 to 18 % at week 52, and by 13 and 10 % respectively at week 78. Food consumption was similar across all groups.

Haematological investigations revealed no treatment-related changes in any dose group at either week 52 or 78. Thyroid function analysis revealed no treatment-related changes to T_3 or TSH levels in any dose group at either week 52 or 78. However, T_4 levels were statistically significantly reduced in both males and females at 1000 ppm both at 52 weeks (by 56 %) and at 78 weeks (by 25 %).

At post-mortem there were no macroscopic or organ weight changes that could be attributed to mancozeb at either 52 or 78 weeks. Histopathological examination revealed no statistically significant evidence of any effects (non-neoplastic or neoplastic) attributable to mancozeb treatment in any dose group at either 52 or 78 weeks.

The results of this study indicate that mancozeb was not carcinogenic in mice following 78-week oral administration. A NOAEL for systemic toxicity of 100 ppm (equivalent to 13 mg kg^{-1} for males and 18 mg kg^{-1} for female mice respectively) can be identified from this study. At 1000 ppm (equivalent to 131 mg kg^{-1} for males and 180 mg kg^{-1} for females respectively), there was evidence of a significant reduction in serum T_4 levels.

3.3.6.2.2.3 Ethylene Thiourea

A published paper that briefly summarises the results from the testing of more than 100 different substances for carcinogenicity in mice, as part of the USA National Cancer Institute Testing Programme in the 1960's is available (Innes *et al*, 1969). Very little information is given for each individual study. Eighteen mice per sex (aged 7 d) from each of two different strains (C57BL/6 and C3H), received ETU (purity unstated) at 215 mg kg^{-1} in 0.5 % aqueous gelatin by oral gavage on days 1 - 21 followed by 646 ppm in the diet from day 21 until termination at 18 months. The dose was reported as being the maximum tolerated dose for infant and young mice but not necessarily adults (no further details given). At study termination 14 male and 18 female C57BL/6, and 18 male and 13 females C3H mice survived to study termination. Negative and positive control groups were also included in the study. An increase in the incidence of hepatomas (14/14 male and 18/18 female C57BL/6 mice and 18/18 male and 9/13 female C3H mice) following ETU administration is indicated, compared with negative controls (8/73 male and 0/83 female C57BL/6 mice and 5/89 male and 1/75 female C3H mice.

Overall, the data indicate that ETU administered orally to mice leads to an increase in the incidence of liver tumours, in strains of mice with a higher background incidence of such tumours. These findings are of doubtful significance for human health. However, not withstanding this the report is too limited to enable any firm conclusions to be drawn.

A complex NTP study designed to assess the carcinogenic effects of ETU administered perinatally (via dams diet), to adults only, and combined prenatal and adult exposure, for 2 years in C57BL/6N mice is available. Groups of 50 females received ETU (99 % purity) continuously in the diet at concentrations of 0, 33, 110 or 330 ppm for 2 weeks prior to mating, throughout gestation and lactation (dose selection study, see Section 3.3.7.1.3).

Dams were mated with unexposed males. On day 7 postpartum litters were reduced to a maximum of 8 pups. Post-weaning (day 28 postpartum) pups were separated by sex within maternal dose groups, (including the controls), and at week 8 animals were grouped, 60 animals per sex per group, and exposed to ETU concentrations of 0, 100, 330 or 1000 ppm for up to 2 years (dose selection study, see Section 3.3.4.1.1.3). The final dosing regimens for the respective groups over 2 years were; 0:0 for overall control animals (these animals received no ETU exposure throughout life), 330:0 for perinatally only exposed animals (exposed during gestation and lactation to weaning), 0:330 or 0:1000 for adult only exposed animals (these animals were not exposed *in utero* or during lactation), and 33:100, 110:330, 330:330 and 330:1000 ppm for combined perinatal and adult exposed animals (these animals received ETU throughout life). The mean intake of ETU in terms of mg per kg body weight could not be calculated as food consumption data were not presented (Unpublished, 1989b).

An additional interim-sacrifice group of 10 rats per sex per group received these doses for 9 months only.

Throughout the study the animals were observed for clinical signs of toxicity and body weight and food consumption was monitored. At interim sacrifice blood and urine samples were taken for haematological, clinical chemistry and urinalysis. At both interim and terminal sacrifice blood samples were taken for thyroid function analysis (T_3 and TSH levels, T_4 levels were not measured). All animals at interim sacrifice, and decedents and survivors of the full study were necropsied and standard organ weights recorded. Extensive histopathological assessment was conducted on all animals.

All animals survived to the interim sacrifice. Survival rates at study termination were comparable to that of the 0:0 overall control group, for all animals across the different exposure regimens. No clinical signs of toxicity were observed in any groups throughout the study. Food consumption in all animals was comparable with controls throughout the study.

At terminal sacrifice the mean body weight gains were reduced in males by 14 %, in exposure groups 0:1000 and 330:1000 ppm, compared with overall controls. Mean body weight gains were reduced in females (18-30 %), compared with overall controls, in all exposure groups except 330:0 ppm. No other changes in body weight were reported in any other animals across the remaining exposure regimens.

At interim sacrifice absolute liver weights were statistically significantly increased, compared with controls in both males (by 14-23 %) in all exposure groups except in 330:0 and 0:330 animals, and in females (by 25-41 %) in all exposure groups except animals at 330:0 ppm. Liver weights in these residual groups were comparable with controls. Absolute thyroid weights of males and females of the 0:1000 and 330:1000 were reportedly increased, but not statistically significantly (no numerical details presented). No treatment-related changes in other body organ weights of any exposed animals at interim sacrifice compared with controls were reported. No information regarding organ weight changes at study termination was reported. No treatment-related changes in any dose group in haematology, clinical chemistry or urinalysis compared with controls, were reported.

Thyroid function analysis of interim killed animals, revealed a statistically significant increase in T_3 levels in males (25 %) and females (34 %) of the 330:1000 ppm group, and females (40 %) of the 0:1000 ppm group, compared with overall controls. Levels in other exposure groups were comparable with controls. TSH levels were statistically significantly

increased in males of the 330:330 and 330:1000 ppm groups (28 and 38 %, respectively) compared with overall controls. Levels in males of other exposure groups and all females were comparable with overall controls.

At terminal sacrifice T_3 levels were statistically significantly increased in males by 72 and 51 % and in females by 22 and 5 % of groups 330:330 and 330:1000 ppm, respectively, compared with overall controls. T_3 levels in other groups were comparable with overall control levels. TSH levels were increased in a dose-related manner, in males (35 to 860 %) and females (26 to 1000 %) of all exposure groups, compared with overall controls. Increases in TSH were statistically significant in males of the 0:1000 and 330:1000 ppm groups only, and in females of the 0:1000, 110:330, 330:330 and 330:1000 ppm groups, only.

Histopathological examinations of interim killed animals revealed changes in the thyroid gland and liver. Thyroid gland changes were an increased incidence of diffuse cytoplasmic vacuolisation of the follicular epithelium in all males and females of all exposure groups except those exposed perinatally only (330:0 group) and the overall control group, which had a zero incidence. Thyroid follicular cell hyperplasia was also observed in 2 females of the 0:1000 ppm group only, compared with 0 in the 0:0 control group. No evidence of thyroid hyperplasia was observed in other animals at other exposures.

Liver changes observed were: centrilobular hepatocellular hypertrophy in 5-10 males of groups 330-330, 110:330, 0:1000 and 330-1000 ppm, and in 9-10 females of groups 330:1000 and 0:1000 ppm (livers of females of groups 330:0, 330:330 and 110:330 were not examined), animals of all remaining groups (0:0, 330:0 and 0:330 ppm) had a 0 incidence; and eosinophilic foci in the liver in 4-5 females of groups 0:1000 and 330:1000 ppm groups, animals of all remaining groups (0:0, 330:0 and 0:330 ppm) had a 0 incidence.

In animals killed at study termination the incidence of follicular cell hyperplasia ranged between 6 and 96 % in males, with the lower incidence being in the 110:330 ppm for males, and between 26 and 94 % in females with the lower incidence being in the 0:330 ppm exposure group, compared with 0 and 4 % in the respective 0:0 control groups, and in males of the 0:330 ppm group. A 94 % incidence of vacuolisation of the thyroid gland was also observed in males and females of the 0:330 and 0:1000 ppm, exposure groups, compared with 0 and 6 % in controls. No data for other exposure groups was presented. Hepatocellular hypertrophy was reported in adult only exposure groups (0:330 and 0:1000 ppm) at an incidence of 72 and 50 %, respectively in males, and 22 and 0 %, respectively in females.

At terminal sacrifice, only, hyperplasia of the pituitary gland Pars distalis was reported. The incidence of effects was significantly increased over control levels in males and females in the 0:1000 and 330:1000 ppm exposure groups (78 and 64 % in males, compared with 0:0 control incidence of 0 %, and 55 and 60 % in females, compared with 0:0 control incidence of 40 %). Incidences in other exposure groups were 2 to 5 % in males and 38 to 48 % in females.

At interim sacrifice hepatocellular adenomas were observed in 2 males and 2 females at 0:1000 ppm, in 1 male of the 330:0 ppm and in 1 male and 1 female at 330:1000 ppm groups, compared with 0 in overall controls and other exposure groups. At terminal sacrifice the incidence of hepatocelluar adenomas in males ranged from 31 to 41 %, with the lower incidence being in the 110:330 and 330:1000 ppm groups, compared with incidences of 23 % in overall controls and 18 % in the 0:1000 ppm group. In females incidences ranged from 28

to 69 %, with the lower incidences being in the 0:1000 and 330:1000 ppm exposure groups, compared with 4 % in the overall control groups.

At terminal sacrifice the incidence of hepatocelluar carcinomas in males ranged from 32 to 96 % in males, with the lower incidence being in the 0:330, 110:330 and 330:330 ppm groups, compared with incidences of 27 % in overall controls. In females incidences ranged from 46 to 98 %, with the lower incidences being in the 0:330, 110:330 and 330:1000 ppm exposure groups, compared with 4 % in the overall control groups. An increased incidence of hepatobalstomas in males (6/50) and females (2/50) of the 0:1000 ppm exposure group compared with the 0:0 and 0:330 ppm groups (0 % and 0-2 %) is reported. No data regarding effects in other exposure groups were reported.

At terminal sacrifice, follicular cell adenomas were observed in males (52 and 67 %) of groups 0:1000 and 330:1000 ppm, respectively. Incidences in males of other exposure groups were 2 %, compared with 0 in overall control males. Follicular cell adenomas were observed in females in the majority of exposure groups, the incidence of tumours ranging from 10 to 76 %, compared with 0 in overall control animals and 2 % in females of the 0:330 ppm exposure group. The lower incidences of females' findings (10 to 20 %) were observed in the 110:330 and 330:330 ppm exposure groups. At terminal sacrifice, follicular cell carcinomas were observed in males and females of the 0:1000 and 330:1000 ppm exposure groups (10 and 71 % in males and 16 and 76 % in females, respectively, compared with 2 and 0 % in 0:0 controls). Incidences in males and females in other exposure groups were comparable with 0:0 overall controls.

At terminal sacrifice an increased incidence of pituitary gland adenoma was reported in males of the 0:1000 and 330:1000 ppm exposure groups (20 and 10 %, respectively), compared with a 0 % incidence in 0:0 controls and other exposure groups. In females the incidence of pituitary gland adenoma was high across the exposure groups, including the 0:0 control group, the relative incidences being; 21, 39, 53, 29, 55 and 51 % for the 0:0, 0:330, 0:1000, 110:330, 330:330 and 330:1000 ppm exposure groups.

No other neoplastic or non-neoplastic effects that could be directly related to ETU exposure were observed. Lung alvelor/bronchiolar adenomas and carcinomas were observed in males from all exposure groups, with incidences ranging from 12 to 25 %, compared with 4 % in 0:0 overall controls, in strains of mice with a higher background incidence of such tumours. These findings are of doubtful significance for human health.

Overall, the results of this study indicate that ETU administered orally either perinatally, only as an adult or from birth onwards induced a significant increase in the incidence of: thyroid follicular cell hyperplasia and thyroid hormone levels (T_3 and TSH) and in thyroid follicular cell adenomas and carcinomas; liver hypertrophy and hepatocellular adenomas and carcinomas; and pituitary gland Pars distalis hyperplasia and adenomas, compared with untreated controls.

ETU was found to induce thyroid tumours in rats and mice in the submitted studies. This substance is well known and documented to be capable of inducing thyroid tumours in rodents (IPCS, 1988).

3.3.6.3 DERMAL

3.3.6.3.1 Mouse

3.3.6.3.1.1 Zineb

No data are available.

3.3.6.3.1.2 Mancozeb

In a skin painting study groups of 20 female Swiss mice received no-treatment, vehicle (DMSO or Acetone), 3,4 benzo[a]pyrene (as positive control substance), or 100 mg kg^{-1} mancozeb (technical grade >95 % purity, remaining composition not reported) in DMSO three times a week for 60 weeks (Shukla *et al*, 1990). Animals were inspected weekly for any clinical signs of toxicity and were weighed every 2 weeks. At the end of the study period (60 weeks) all surviving animals were killed and examined both macro and microscopically (details of the tissues and organs examined were not reported).

Deaths were observed in the mancozeb treated animals from week 17 (data presented graphically only). Fourteen of the 20 mancozeb treated animals apparently survived to week 54 of the study and less than 6 animals survived until the end of the study. A 16 % reduction in body weight (data presented graphically only) of mancozeb treated animals was reported from week 8 of the study. All other groups had comparable body weight gains throughout the study. Clinical signs of toxicity in mancozeb-treated animals were lethargy, anorexia (decreased food consumption and progressive weight loss), scaly skin and baldness. These effects became significant after week 30, with dermal thinning and complete disappearance of the subcutaneous fat layer occurring at weeks 50-52.

Neoplastic and non-neoplastic lesions confined to the treated area of skin were reported in mancozeb treated animals (exact numbers of animals affected not stated). Non-neoplastic lesions including keratinized skin were reportedly observed from week 1 to the end of the study period, in the majority of animals. Tumours were observed in 5/14 (35 %) of mancozeb treated animals, compared with 12/12 (100 %) in positive control animals, and 0/16 and 0/17 in acetone and DMSO vehicle controls, respectively. The tumours were observed from week 31 and presented as finger-like or papillomatous growths on the treated areas. Histopathological examination revealed that the tumours observed included; squamous cell papillomas (flat and pedunculated), keratoacanthomas and mixed type tumours, all of which were benign in nature.

Due to the high rate of mortalities occurring in this study and limited information regarding the extent of examination no firm conclusions can be drawn with regard to the tumourigenic potential of mancozeb via the dermal route.

A limited summary of a study is presented in a secondary literature source (IARC, 1976). Groups of male and female mice (18/sex) aged 28 d from two different strains (C57BL/6 C3H and AKR hybrids) were treated with a single subcutaneous injection of 1000 mg kg^{-1} zineb (97 % purity) in 0.5 % gelatine and observed for 78 weeks. At study termination 16 and 18 males and 18 and 17 females of each strain respectively, had survived. An increase in the incidence of systemic reticulum-cell sarcomas was observed in male C57BL/6 C3H mice,

5/18 (28 %) compared with 8/141 (6 %) in controls. There was no other increase in tumour incidence in any other animals compared with controls. No historical control data were reported. No details of other observations were reported. Overall considering the route of exposure, the low number of test animals and the fact that only one dose level was used no conclusions regarding the significance of this finding can be made.

3.3.6.4 INHALATION

No data were submitted or are available.

3.3.6.5 SUMMARY OF CARCINOGENICITY

There are no carcinogenicity studies available of an adequate quality or standard investigating the carcinogenic potential of zineb via any route of exposure. Consequently, no conclusions regarding the carcinogenic potential of zineb can be made based directly on the available zineb data. However, based on similarities in toxicokinetics and toxicodynamics it is predicted that zineb would show a similar, qualitative and quantitative carcinogenicity profile to the structurally related mancozeb. Furthermore, studies are also available on the principal metabolite of both zineb and mancozeb, ETU. There are no inhalation or adequate dermal carcinogenicity studies available for mancozeb. Based on toxicokinetic information it is possible to predict that the carcinogenic potential of zineb (and mancozeb) in rodents via inhalation, will be similar to that observed via oral administration. However, it is not possible to quantify the levels at which a response would occur. Toxicokinetic data also demonstrate negligible dermal absorption of both aqueous zineb and mancozeb (<1 %) and therefore it can be predicted that neither zineb nor mancozeb are likely to be carcinogenic via the dermal route of exposure.

Two well-conducted oral studies in rats with mancozeb are available, the results of which indicate that mancozeb induces thyroid gland follicular cell adenomas and carcinomas in rats following 24-month dietary administration at concentration of 750 ppm (equivalent to 31-40 mg kg^{-1} d^{-1}). No tumours were observed in mice, in a well-conducted oral study with mancozeb at doses of up to 1000 ppm (equivalent to 13-18 mg kg^{-1} d^{-1}). This latter observation is of some significance since ETU was found to induce both thyroid and liver tumours in mice at doses of 1000 ppm and above. Toxicokinetic data indicate that in the mouse ETU is a minor metabolite following oral administration. Therefore, it is likely that the levels of ETU available to the mouse via metabolism of mancozeb in this study were not sufficiently high enough to produce any effects. It is probable that at doses high enough in mice to result in the production of significant levels of ETU then carcinogenic effects might occur. However, from the data available this would seem to be at dose levels significantly above those seen to produce effects in the more sensitive species, the rat. Since the thyroid was the key target tissue for both non-neoplastic and neoplastic changes in the rat, this is considered to be the principal tissue of concern with respect to carcinogenic activity of mancozeb, and thus by analogy, zineb.

In view of the lack of genotoxic activity of mancozeb (and zineb), it is concluded that the thyroid tumours seen in rats arose as a result of the chronic hyperplastic response of the thyroid follicular cells, induced by a direct action of mancozeb or more likely its metabolite ETU. ETU was found to induce thyroid tumours in rats and mice in the submitted studies. This substance is well known and documented to be capable of inducing thyroid tumours in rodents. It is believed that ETU exerts this effect through a non-genotoxic mechanism involving the disruption of thyroid hormone homeostasis by inhibition of thyroid peroxidase

leading to a reduction in the production of T_4 and hence a reduction in circulating levels of T_4. This in turn leads to an increase in trophic stimulation (increased secretion of TSH by the pituitary) of the thyroid. This may lead to a hypertrophic and hyperplastic response in the case of long-term thyroxine deficit and continued trophic stimulation. The hyperplastic state is believed to lead to an increased potential for neoplastic change and tumour development. Such a mechanism is considered to be of possible relevance to human health, though rodents, particularly the rat, may be more responsive because of the inherently normal higher activity of the thyroid. Furthermore, because of the presence of thyroid binding globulin in humans that can act as a reserve for thyroid hormone, any extrapolation of data from rat to human is rendered more uncertain. The effects which signal this proposed sequence of events (decreased T_4, increased TSH, thyroid follicular cell hypertrophy and hyperplasia) are seen as a consequence of exposure to zineb and more clearly for mancozeb. In reviewing all the information available these changes can be seen to be related both to the dose of substance administered and the duration of the exposure. It is likely that the effects seen with zineb and mancozeb are due to the presence of ETU in the test animals, most probably as a consequence of metabolism. There is also the possibility of an additional contribution of low level ETU contamination in the test material.

Whatever the actual contribution from these potential sources, it is probable that the long-term exposure of the test animals to ETU at a high enough dose level of the parent compound leads to an inhibition of thyroid hormone production and ultimately to tumour formation via the mechanism described. Since, from the limited toxicity data available and taking into account the toxicokinetic information, zineb is toxicologically similar to mancozeb it is predicted that at high enough doses zineb would induce similar neoplastic changes, including the induction of tumours, in the thyroid. Since the mechanism outlined above is likely to have a threshold (i.e. the underlying inhibition of thyroid peroxidase) then a long term NOAEL for the effects on the thyroid should also be, in principle, a NOAEL for potential neoplastic change and tumour formation. For mancozeb, the lowest NOAEL for chronic oral administration in the most sensitive species, the rat, is ~5 mg kg^{-1} d^{-1}. It is likely that a similar value would be appropriate for zineb. In relation to the liver tumours produced in mice by ETU, as indicated above, studies on mancozeb resulted in no effects in the liver of mice at doses of up to 13-18 mg kg^{-1} d^{-1}. This is clearly above the NOAEL for thyroid effects and thus if the liver was a potential target tissue for zineb in the mouse this would be at dose levels likely to be already associated with concerns for thyroid carcinogenicity.

3.3.7 EFFECTS ON REPRODUCTION

3.3.7.1 EFFECTS ON DEVELOPMENT

3.3.7.1.1 Zineb

3.3.7.1.1.1 Oral

3.3.7.1.1.1.1 Rats

Groups of 22-26 pregnant rats (strain unspecified) received approximately 0, 200, 632, or 2000 mg kg^{-1} d^{-1} zineb formulation (stated by the Authors to be a formulation of zineb with

analytical results indicating approximately 85 % as EBDC, 0.35 % ETU, other constituents unknown) in corn oil by oral gavage daily on days 6-20 (Unpublished, 1980). The day after the last treatment Caesarean section was performed. Uterine horns were examined with respect to the number and position of live, dead, and resorbed fetuses. One half of the live fetuses were examined for soft tissue and external anomalies, and the remaining fetuses for skeletal anomalies.

There was no indication of whether or not clinical signs of toxicity were observed, although there were no maternal deaths. Group mean maternal body weight gain was not affected amongst dams receiving up to 632 mg $kg^{-1}d^{-1}$ when compared to controls. However, at 2000 mg $kg^{-1}d^{-1}$ weight gain was reduced by approximately 20 %. Similarly, maternal food consumption was not adversely affected at up to 632 mg $kg^{-1}d^{-1}$ but at 2000 mg$kg^{-1}d^{-1}$ was reduced by approximately 15 %.

There were no significant treatment-related effects on the number of live litters (25, 24, 20, and 22 amongst animals receiving 0, 200, 632, and 2000 mg $kg^{-1}d^{-1}$ respectively). Amongst the live litters, the number of implants per dam was 11.0, 10.2, 11.3, and 12.4 for each of the respective groups. There were no dead fetuses observed and, expressed in relation to the number of implants per dam only, no adverse effects on the percentage of early or late resorptions (9 %, 9 %, 4 %, 9 % and 6 %, 15 %, 16 %, 5 % respectively). Amongst live litters, the sex ratio was 58 %, 46 %, 55 %, and 48 % males at 0, 200, 632, and 2000 mg $kg^{-1}d^{-1}$ respectively. Fetal body weight was slightly reduced at 2000 mg $kg^{-1}d^{-1}$ (by approximately 8 % compared to controls, but attaining statistical significance).

There were exposure-related increases in the incidence of a small number of skeletal anomalies. The mean percentage, expressed on a litter basis, of raised cranium was 0, 0.8, 0 and 2.8 % amongst animals that had received 0, 200, 632, and 2000 mg $kg^{-1}d^{-1}$ respectively. Short tail 0, 0, 0.4 and 10.9 %; and kinked tail 0, 0, 0.4, 16.2 %. There was a clear increase in the incidence of lateral hydrocephaly (described as an anomaly rather than as a major abnormality) in the pups from dams that had received 2000 mg $kg^{-1}d^{-1}$ (0, 0, 0, 26.3 %).

Overall, limited reporting makes it difficult to comment definitively about maternal toxicity, although 2000 mg $kg^{-1}d^{-1}$ was an exposure level associated with reductions in body weight and food consumption. Apparently, no significant maternal or developmental effects occurred as a result of exposure of pregnant dams to up to 632 mg $kg^{-1}d^{-1}$. Developmental effects (hydrocephaly) occurred in rat pups as a result of dams receiving 2000 mg $kg^{-1}d^{-1}$ technical zineb. Other effects seen in pups at 2000 mg $kg^{-1}d^{-1}$ (e.g. skeletal anomalies, slight reduction in pup weight) are of doubtful toxicological significance.

Groups of 3-14 pregnant female rats (strain not specified) received a single oral gavage dose of 0, 1000, 2000, 4000, or 8000 mg kg^{-1} aqueous zineb ('technical grade'; no further details on purity or composition given) on day 11 or 13 of gestation (Petrova-Vergieva and Ivanova-Chemishanska, 1973). Subsequently, groups of 9-12 pregnant female rats received repeated daily doses of 0, 125, 250, 500, or 1000 mg kg^{-1} aqueous zineb on days 2-21. On day 21, following Caesarean section, investigations included counts of corpora lutea, implantation sites, live or dead fetuses and a visual assessment of any external malformations. Skeletal examination using alizarin red staining was performed on approximately two-thirds of the offspring (exact numbers unclear).

No information was presented on maternal toxicity although the highest exposure level of 8000 mg kg^{-1} was stated to be two-thirds of the LD_{50} for these animals.

In relation to the single dose aspect of the study, for animals that had received a single oral dose of zineb on day 11 there were no effects on the mean number of corpora lutea per animal compared to controls (about 8-10 per animal). There were no significant effects on the mean number of implantations (about 8-9 per animal). At 4000 and 8000 mg kg^{-1} there were statistically significant decreases in the mean number of live fetuses (8.1/dam in controls, then 9.2, 8.0, 6.4 and 4.7 at 1000, 2000, 4000 and 8000 mg kg^{-1} respectively). Similarly, the mean number of late deaths was increased at 4000 and 8000 mg kg^{-1} (0 in controls, and at 1000 and 2000 mg kg^{-1} but approximately 1 and 3 late deaths per dam at 4000 and 8000 mg kg^{-1}). There were no resorptions observed in any group except for a marked increase at 4000 mg kg^{-1} (about 1.4 per dam).

There were no gross malformations observed at 0 or 1000 mg kg^{-1}. However there was a statistically significant, dose-related increase in the mean total number of animals showing gross malformations (0.6/dam, 3.3/dam, 4.7/dam respectively) and in the specific types of malformation seen at 2000 mg kg^{-1} and above. The malformations seen were severe, with a marked lack of ossification in skull bones, vertebrae and long bones being the main features. In addition, micrognathia, phocomelia (fore and hind limbs), encephalocoele, and hydrocephaly were observed. A similar pattern of malformations was seen for animals receiving a single dose of zineb on day 13; numerically, there were more skeletal malformations, although only animals receiving 4000 mg kg^{-1} or more were affected.

Overall, for animals receiving a single dose of zineb, there were no abnormalities seen at 1000 mg kg^{-1}. At 2000 mg kg^{-1} or more, effects were marked with the key features being impaired skeletal development and an increase in the number of late fetal deaths.

Fewer details were presented in relation to the repeated exposure study, but there were no effects on the number of corpora lutea, implantations, live fetuses, resorptions, late deaths and no malformed fetuses observed. Overall, repeated administration of up to 1000 mg kg^{-1} zineb between days 2-21 of gestation was without adverse effect on the development of offspring.

In another investigation, no significant adverse effects were noted in any of the parameters recorded in a study designed to investigate potential post-implantation effects in which groups of 5-6 pregnant female Sprague-Dawley rats received approximately 0, 10, 21, 31, 43, or 50 mg kg^{-1} zineb (99.9 % pure) by dietary administration through days 6-15 of gestation or days 6-9 for pseudopregnant animals (Sing and Spencer, 1981). Animals were sacrificed on day 16; investigations were limited but included removal of ovaries and uteri for the determination of total protein and glycogen, implantations and resorptions, numbers of live and dead fetuses, and pup weights. There was no other physical examination of pups. Overall, although no adverse post-implantational effects were reported up to 50 mg kg^{-1} received during gestation, the limited and unusual investigations limit the value of this study.

As part of the same unconventional study reported by Ryazanova (1967) in which male and female rats received 10 or 100 mg kg^{-1} d^{-1} zineb by oral gavage for up to 6 months there was some suggestion of developmental effects (early fetal death, resorptions, reduced body weight and early mortality of pups, the observation of ‘kinked tails’). However, it remains

impossible to determine the significance of such comments due to poor reporting, and no conclusions can be drawn from this report.

3.3.7.1.1.1.2 Mice

Groups of 23-26 pregnant CD-1 mice received approximately 0, 200, 632, or 2000 mg kg^{-1} d^{-1} zineb formulation (stated by the Authors to be a formulation of zineb with analytical results indicating approximately 85 % as EBDC, 0.35 % ETU, other constituents unknown) in corn oil by oral gavage daily on days 6-18 (Unpublished, 1980). The day after the last treatment Caesarean section was performed. Uterine horns were examined with respect to the number and position of live, dead, and resorbed fetuses. One half of the live fetuses were examined for soft tissue and external anomalies, and the remaining fetuses for skeletal anomalies.

There was no indication of whether or not clinical signs of toxicity were observed, although there were no maternal deaths. Maternal body weight gain and food consumption were not adversely affected by treatment.

There were no significant effects on the number of live litters (26, 22, 26, and 22 amongst animals receiving 0, 200, 632, and 2000 mg $kg^{-1}d^{-1}$ respectively). Amongst the live litters, the number of implants per dam was 12.3, 11.6, 11.9, and 11.6 for each of the respective groups. There was only one dead fetus observed (at 200 mg $kg^{-1}d^{-1}$) and, expressed in relation to the number of implants per dam only, no adverse effects on the percentage of early or late resorptions (6 %, 5 %, 6 %, 6 % and 3 %, 1 %, 2 %, 2 % respectively). Amongst live litters, the sex ratio was 75 % (which seems unusually high), 45 %, 46 %, and 43 % males at 0, 200, 632, and 2000 mg $kg^{-1}d^{-1}$ respectively. Pup body weight was not adversely affected.

There were no significant exposure-related increases in the incidence of skeletal anomalies. There was an isolated occurrence of slight 4^{th} ventricle hydrocephaly in the pups from one litter of dams that had received 2000 mg $kg^{-1}d^{-1}$. Otherwise, there were no significant effects on the soft tissues examined.

Overall, limited reporting makes it difficult to comment conclusively about maternal toxicity, although apparently, no significant maternal effects occurred as a result of exposure of pregnant dams to up to 2000 mg $kg^{-1}d^{-1}$ technical zineb. The isolated occurrence of hydrocephaly at 2000 mg $kg^{-1}d^{-1}$ is probably of little toxicological importance; although it is consistent with effects seen in other developmental studies. However, in this study it has been categorised as an anomaly and so probably represents an effect of little structural significance. Hence, it seems reasonable to conclude that no adverse developmental effects occurred as a result of exposure of pregnant mice to up to 2000 mg $kg^{-1}d^{-1}$ technical zineb.

3.3.7.1.1.2 Dermal

No studies are available.

3.3.7.1.1.3 Inhalation

Only limited data are available.

As part of a series of developmental studies, groups of 4-6 pregnant female rats (strain not specified) were exposed by inhalation (it was unclear whether this was whole-body or nose-

only) to 0 or 100 mg m^{-3} zineb ('technical grade'; no further details on purity or composition given) 4 h d^{-1} from either day 4, 5, 6, or 7 until day 21 of gestation (Petrova-Vergieva and Ivanova-Chemishanska, 1973). It was not stated if zineb was administered as a powder or if it was in solution or suspension, and there was no information on particle/droplet size. Animals were allowed to deliver and pups were maintained and weighed at weekly intervals for at least one month post-partum, although no information was presented in relation to the general status of the dams. Embryo viability, litter size, birth weight and survival were recorded.

There were no adverse effects on the mean total number of corpora lutea (9.2, 9.8, 10.5, 9.3), implantations (9, 9.6, 10, 8.8), live births (8.8, 9.6, 10, 8.5), dead fetuses (0.17, 0, 0, 0.33) and there were no fetuses with malformations. Given that the dams were allowed to deliver and presumably would have been required for weaning pups, it is unclear how some of these parameters were measured (e.g. corpora lutea). Overall, there was no evidence of developmental effects in rats exposed up to 100 mg m^{-3} zineb, 4 h d^{-1} during the major period of organogenesis, although there are limitations in reporting and no information as to whether or not the material was respirable. Hence, it is difficult to draw any firm conclusions from this study, on the potential of zineb to induce developmental effects.

3.3.7.1.2 Mancozeb

3.3.7.1.2.1 Oral

3.3.7.1.2.1.1 Rats

In an unpublished study conducted in accordance with OECD guidelines, conforming with GLP, groups of 25 pregnant female Crl:CD rats received oral gavage doses of 0, 10, 60, or 360 mg kg^{-1} mancozeb (89 % pure; other components not stated) suspended in aqueous methylcellulose on days 6 to 15 of gestation. Animals were killed on day 20 for examination of uterine contents. Numbers of corpora lutea, implantation sites, resorption sites, live and dead fetuses were recorded. All fetuses were weighed, approximately half taken for skeletal examination and half for visceral examinations (Unpublished, 1988e).

At 360 mg kg^{-1}, one dam was killed due to poor condition and four other dams also exhibited clinical signs of toxicity (unsteady gait, and loss of use of hind limbs). At 360 mg kg^{-1} there were statistically significant reductions in food consumption (approximately 15 % less than control) and body weight gain (approximately 30 % less than control) during the treatment period. There were no clinical signs of toxicity and no adverse effects on body weight and food consumption at 10 or 60 mg kg^{-1}. There were no macroscopic pathology abnormalities seen amongst the dams from any of the treated groups.

There were no adverse effects on corpora lutea (16.3, 15.9, 16.6, 15.7 per dam), implantations (15.0, 15.0, 15.0, 15.1), live young (14.3, 13.8, 13.9, 14.0), early or late resorptions (0.7, 1.1, 1.0, 1.0 and 0, 0.1, 0.04, 0.1), and hence no effects on pre- or post-implantation loss. Mean fetal weight (3.4 g, 3.4 g, 3.4 g, 3.3 g) and crown rump length (3.5 cm, 3.5 cm, 3.5 cm, 3.4 cm) were not adversely affected.

There were no significant visceral or external abnormalities seen in fetuses. However, at the highest exposure level, 360 mg kg^{-1}, there was a slight increase in the observation of two

skeletal anomalies: reduced ossification of the interparietal bone (76 %, 80 %, 87 %, 90 %), and an increase in the size of the anterior fontanelle (2 %, 0.6 %, 4 %, 12 %). In addition, reduced ossification of thoracic vertebral centra (28 %, 39 %, 39 %, 51 %) was seen amongst all treated groups, although only attaining statistical significance at the highest exposure level. As the extent of reduced vertebral ossification amongst all of these exposed groups was still within the historical control range, and this finding evidently occurs at a high spontaneous level, the toxicological significance, particularly at 10 and 60 mg kg^{-1} is doubtful.

Overall, this study indicates that developmental effects (skeletal anomalies) are only seen following oral administration of 360 mg kg^{-1} or more during the gestation period of the rat, a level at which there was maternal toxicity (severe clinical signs of toxicity, reduced body weight gain and even mortality). No maternal toxicity and no significant developmental effects were seen at exposures of 60 mg kg^{-1} or less.

3.3.7.1.2.1.2 Rabbits

In a study available only in abstract form, groups of 20 pregnant female New Zealand White rabbits received oral gavage doses of 0, 10, 30, or 80 mg kg^{-1} mancozeb (unstated purity and composition) suspended in methylcellulose (Solomon and Lutz, 1989) on days 7-19 of gestation. On day 29, the does were killed and examined, and fetuses were examined for external, visceral and skeletal abnormalities.

At 80 mg kg^{-1} two does died, there were 5 litters aborted, and unspecified clinical signs of maternal toxicity were apparent. At lower dose levels there were no adverse effects reported with respect to clinical signs of toxicity, body weight gain, and food consumption in does and there were no adverse effects claimed for female reproductive parameters (although it was unclear what was investigated).

Apparently, there were no malformations or variations in pups from any of the treated groups. Overall, although this brief report indicates maternal toxicity at 80 mg kg^{-1}, no adverse maternal effects at 30 mg kg^{-1} or below, and no evidence of developmental effects in pups, it is difficult to draw any firm conclusions due to the limited extent of information available.

3.3.7.1.2.2 Dermal

No data are available.

3.3.7.1.2.3 Inhalation

Groups of 27 pregnant female Crl:CD rats were exposed, whole-body, 6 h d^{-1} to 0, 1, 17, or 55 mg m^{-3} mancozeb dust (80 % pure, 20 % unspecified ‘inert’ ingredients) on days 6-15 of gestation (Lu and Kennedy, 1986). A preliminary study was conducted using exposures up to 1890 mg m^{-3} but this was cut short due to excessive mortality (further details of this study are reported below). Although not measured in the main study due to the low concentrations used, the median mass aerodynamic diameter of mancozeb dust particles in the preliminary study was determined to range from 1.4-6.4 µm (with ‘most’ [numerically] in the range 1.4-1.9 µm). Dams were weighed at approximately weekly intervals, killed on day 21 of gestation, and ovaries and uterus removed and examined. The number of corpora lutea was counted, as was the number of implantation sites, number and location of live and dead fetuses, and resorption sites. Fetuses were weighed and examined macroscopically for any

external malformations; subsequently about half were selected for skeletal, and half for visceral examinations.

There were no maternal deaths in the main study. At 55 mg m^{-3}, hind limb weakness and slower righting reflexes were observed in 6/27 dams compared to controls and there was a statistically-significant reduction in body weight gain (40 % lower than control). The dams from other groups were not adversely affected. There were no significant differences in the number of females pregnant in each group or in the number of live litters (26/27, 23/27, 24/27, 24/27 for both of these parameters). There were no significant differences in the mean number of corpora lutea per dam (13.7, 11.7, 12.5, 12.1), in the mean number of implants (11.0, 9.9, 9.8, 10.0), or in the mean number of resorptions per litter (0.5, 0.3, 0.3, 0.5). Hence, the mean number of live fetuses per litter was unaffected (10.5, 9.7, 9.4, 9.3). In addition, there were no differences in the mean fetal weight per litter (4.2 g, 4.5 g, 4.3 g, 4.2 g).

There were no macroscopic pathology abnormalities seen in dams, and no external, skeletal, or visceral malformations in the pups. Overall, this study indicates that there were no developmental abnormalities resulting from inhalation exposure of dams up to 55 mg m^{-3} (an exposure level associated with some signs of maternal toxicity) during gestation. No adverse effects were reported in pregnant dams exposed to 17 mg m^{-3} during gestation.

The results of the preliminary study were reported in detail and provide useful information in determining the dose-response for developmental effects of mancozeb. Groups of 37-38 mated female Crl:CD rats were exposed, whole-body, 6 h d^{-1} to 0, 110, 890, or 1890/500 mg m^{-3} (exposures reduced from day 2 of exposure due to mortality) mancozeb dust on days 6-15 of gestation (Lu and Kennedy, 1986). Due to excessive mortality and poor general condition the animals in the 890 and 1890/500 mg m^{-3} groups were exposed for only 5 d, but were retained for the remaining study period.

There were 3/37 maternal deaths at 110 mg m^{-3} and a group mean body weight loss. Clinical signs of toxicity in the dams at 110 mg m^{-3} included laboured respiration, hind limb weakness, slow righting reflex. As already indicated, there was excessive mortality and generalised poor condition at the two higher exposure levels used.

No macroscopic pathology abnormalities were seen in dams from the control group or those exposed to 110 mg m^{-3}. 24/27 decedent dams at 890 mg m^{-3} had congested lungs, enlarged adrenals, yellow-white material (possibly the test substance) in the trachea and GI tract, and gaseous distension of the GI tract. These findings were also seen in all decedent animals at 1890/500 mg m^{-3}, although in addition at this exposure level, the GI tract contained sticky, dark-red fluid (presumably blood).

There were no results with respect to the developing offspring from animals exposed to 890 mg m^{-3} due to resorption but there were no statistically significant changes in the incidence of external or visceral malformations amongst the other mancozeb-exposed groups when compared with controls. However, at 110 and 1890/500 mg m^{-3} there were increases in the number of skeletal abnormalities per litter (such as; extra rib, wavy rib, bipartite centra, unossified sternebrae and malaligned sternebrae (0 %, 3 %, 0 %).

Overall, this study indicates that developmental effects (skeletal anomalies) are seen following inhalation of 110 mg m^{-3} or more 6 h d^{-1} during the gestation period of the rat, a

level at which there was frank maternal toxicity. No maternal toxicity and no developmental effects were seen at exposures of 55 mg m^{-3} or less.

3.3.7.1.3 Ethylene thiourea

3.3.7.1.3.1 Rats

In a dose-selection study female F/344 rats (number per group not stated) were continuously administered ETU (purity 99 %) in the diet at concentrations of 0, 8, 25, 83 or 250 ppm (food consumption data were not presented therefore daily intake of ETU can not be determined) for 2 weeks pre mating (with unexposed males) and throughout gestation and up to 9 weeks post-weaning. Exposure concentrations were selected to preclude known developmental effects of ETU. After weaning, on post-partum day 28, selected dams and their offspring (giving final offspring numbers of 10 animals per sex per group) were exposed via the same maternal exposure regimen for 9 weeks (Unpublished, 1989b).

Four pregnant animals from each group were necropsied on day 18 of gestation and examined for any fetal anomalies or changes in the number of implantations, mean number of fetuses per litter, numbers of live or dead fetuses, mean fetal weights, or mean placental weights. At study termination all dams and offspring were necropsied and extensive histopathological examinations of the organs and tissues conducted.

All dams not designated to interim sacrifice survived to study termination. No external gross fetal anomalies or differences in the number of implantations, mean number of fetuses per litter, numbers of live or dead fetuses, mean fetal weights, or mean placental weights were observed in any exposure group at the interim sacrifice on day 18 of gestation.

The number of pups surviving to postnatal day 4 was decreased (by 18 %) in the 250 ppm exposure group, compared to maximum of 4 % in other groups, including controls. Survival of pups from days 4 to 28 and mean body weights on day 28, in all exposure groups was comparable with controls. All weanlings administered ETU in the diet from day 28 survived until study termination.

At necropsy no significant changes in pup body weights compared with controls was observed. Histopathological examination revealed a dose-related incidence of minimal to moderate diffuse thyroid follicular cell hyperplasia in males only (4/10) at 25 ppm and in all males and females at 83 and 250 ppm. One male pup at 83 and 4 males at 250 ppm presented signs of follicular adenoma of the thyroid gland. Pituitary effects (*pars distalis* vacuolization) were observed in 7 male pups at 250 ppm. No abnormal pathological findings were observed in control animals. No other abnormal pathological findings were observed.

Overall, this study shows no obvious effects on development although it has not been investigated in a traditional manner. Some effects (thyroid follicular cell hyperplasia) were seen in the thyroid of pups that had received 25 ppm ETU *in utero* and post-natally.

3.3.7.1.3.2 Mice

In an unconventional study female C57BL/6N mice (number per group not stated) were continuously administered ETU (purity 99 %) in the diet at concentrations of 0, 33, 100, 330 or 1,000 ppm (food consumption data were not presented therefore daily intake of ETU can

not be determined) for 2 weeks pre mating (with unexposed males) and throughout gestation and up to 9 weeks post-weaning. Exposure concentrations were selected to preclude known teratogenic effects of ETU. After weaning, on post-partum day 28, selected dams and their offspring (giving final offspring numbers of 10 animals per sex per group) were exposed via the same maternal exposure regimen for 9 weeks (Unpublished, 1989b).

Four pregnant animals from each group were necropsied on day 17 of gestation and examined for any fetal anomalies or changes in the number of implantations, mean number of fetuses per litter, numbers of live or dead fetuses, mean fetal weights, or mean placental weights. At study termination all dams and offspring were necropsied and extensive histopathological examinations of the organs and tissues conducted.

Ten dams of the 490 assigned to the study (2 in control group, 3 at 33 and 330 ppm, and 2 at 1000 ppm) died before study termination. At the interim sacrifice on day 17 of gestation no external gross fetal anomalies or differences in the number of implantations, mean number of fetuses per litter, numbers of live or dead fetuses, mean fetal weights, or mean placental weights were observed in any exposure group.

The number of pups surviving to post-natal day 7 was not affected by ETU exposure during lactation. The number of pups at 1000 ppm surviving to day 28 post-partum was significantly decreased by 48 %, compared with decreases of 29 to 35 % in other groups, including controls. Three weanlings (group not reported) died before post-natal day 7 (time of death not reported).

At necropsy no significant changes in pup body weights compared with controls was observed. Histopathological examination revealed no effects in animals exposed up to 330 ppm. At 1000 ppm an increased incidence of diffuse thyroid follicular cell hyperplasia in males (7/10) and females (7/10), and of liver centrilobular hypertrophy in males (8/10) and females (7/10) were observed. No other abnormal pathological findings were observed. No abnormal pathological findings were observed in control animals.

Overall, this study shows no obvious effects on development although, like the NTP 1989 rat study, it has not been investigated in a traditional manner. Thyroid follicular cell hyperplasia and liver hypertrophy were observed in pups that had received 1000 ppm ETU *in utero* and post-natally.

In addition to these submitted studies, review documents are available for ETU (IPCS, 1988, Khera, 1987 and BG Chemie, 1990) that give information on published data. In the rat, developmental effects (particularly hydrocephaly, and skeletal malformations) have been demonstrated in the absence of maternal toxicity following a single oral administration of 30 mg kg^{-1} at any time during days 13-20 of gestation, or repeated doses of 5 mg kg^{-1} during days 6-15. At higher exposures (around 200 mg kg^{-1}) mesenchymal necrosis of forelimb buds was noted, still in the absence of maternal toxicity.

3.3.7.2 EFFECTS ON FERTILITY

3.3.7.2.1 Zineb

No conventional studies are available in which the effects of zineb on fertility have been investigated.

As part of a series of unconventional and poorly reported studies, groups of rats (strain, sex and number of rats per group not specified) received oral gavage doses of zineb (purity and vehicle not specified) (Ghizelea and Ozeranschi, 1973). The actual doses administered and frequency of dosing was not clearly indicated. Some organ weights were recorded; the changes claimed included altered uterus and ovary weights. The durations of pro-oestrus, oestrus, and dioestrus were recorded (although it was not clear what had been measured). Apparently, the period of dioestrus was increased and proestrus/oestrus decreased compared to controls although it would seem from the data presented that the values still fell within the range normally seen (as reported in the study). In addition, the mating performance was assessed, although the breeding protocol was unclear. Apparently, there was a reduction in the number of live fetuses, reduced fetal body weight and fertility index at the highest exposure level.

Overall, although this study claims a number of effects on reproductive function and performance, and development of offspring related to zineb exposure, it is impossible to reach any conclusions due to the limitations in study design and reporting.

In an unconventional study reported in limited detail, unstated numbers of male and female rats (strain unspecified) received 0, 10, or 100 mg kg^{-1} d^{-1} zineb by oral gavage (no further details on purity, composition or vehicle given) for up to 6 months (Ryazanova, 1967). The mating regime was unclear but it appears that animals were allowed to breed continuously during this time although some animals were administered zineb for 6 months prior to mating.

No information was provided as to whether or not any systemic effects were observed in males or females. The Authors suggested impaired fertility (delayed pregnancy and 'sterility') but the lack of information regarding investigations performed and poor reporting of results makes it impossible to draw any conclusions.

Mancozeb

In an unpublished two-generation study conducted in accordance with OECD guidelines, conforming with GLP, groups of 25 male and 25 female Crl:CD rats received 0, 30, 120, or 1200 ppm mancozeb (84 % pure; other components not stated) by dietary administration**.** Administration of mancozeb started at the age of 6 weeks, and mating of the first generation (P_1) after 10 weeks of treatment. On day 4 post-partum, pups were culled to leave 5 males and 5 females per litter (the F_{1a} generation). Once the F_{1a} pups were weaned (day 21 post-partum), a second mating of the parental generation (P_1) took place to produce F_{1b} pups. In addition, after a 3-week treatment period (which started when F_{1a} animals were about 3 weeks old) one male and one female F_{1a} pup was selected at random from each litter (a total of approximately 25/group) for mating (becoming the P_2 generation). These P_2 animals produced F_{2a} pups, and once the F_{2a} pups were weaned (day 21 post-partum), a second mating of the P_2 generation took place to produce F_{2b} litters (Unpublished, 1988f).

Comprehensive macroscopic and microscopic pathology examinations were performed on P_1 and P_2 animals (males, 1-2 weeks after siring the second litter, females 1-2 weeks after weaning the youngest litter) and pups that died. However, pups that were killed at the scheduled sacrifices (day 21 post-partum, except F_{2a} pups that were selected for mating) were not examined.

The fixed dietary concentrations of mancozeb corresponded to approximately 2, 7, and 70 mg kg^{-1} d^{-1} and 2, 8, and 80 mg kg^{-1} d^{-1} for P_1 males and females respectively before mating, and about 2, 8, and 80 mg kg^{-1} d^{-1} for P_1 females during gestation (of either F_{1a} or F_{1b} pups). During the lactation phase, but not taking into account the potential for additional mancozeb that may have been transmitted via maternal milk, dietary intake of mancozeb rose to around 4, 18, and 180 mg kg^{-1} d^{-1} for either F_{1a} or F_{1b} pups. Similar dietary intakes of mancozeb were achieved by the P_2 generation.

There were no exposure-related mortalities and no clinical signs of toxicity amongst P_1 or P_2 animals. Statistically significant reductions in body weight gain and food consumption were noted amongst males and females from the P_1 and P_2 generations receiving 1200 ppm mancozeb. For P_1 males body weight gain was 19 % lower than controls during the 10-week pre-mating period, and females, 35 % lower. For P_2 males body weight gain was 6 % lower than controls over a 10-week observation period, and for females, 18 % lower. Food consumption was reduced by around 5-10 %. Food consumption and body weight gain for animals in the other treated groups were not adversely affected.

The number of pregnant P_1 females was not adversely affected by mancozeb treatment (22/25 females from each group, including controls, were pregnant). Mean gestation length was similar between groups (21.4, 21.6, 21.6, 21.7 d). The mean total number of pups delivered (F_{1a}) was not adversely affected (14.1, 12.7, 13.4, 12.5 per litter) and there was no significant increase in the number that were stillborn, or missing/cannibalised during the lactation period. F_{1a} pup viability over the first 21 d post-partum was not affected (98 %, 96 %, 97 %, 98 % survival). The mean pup weight on the day of delivery was 5.8 g, 6.0 g, 6.0 g and 6.0 g and there were no adverse effects on subsequent weight gain. On the day of delivery the sex-ratio of F_{1a} pups was not significantly altered (52 %, 53 %, 52 %, 56 % males).

Similarly, there were no adverse effects on fertility on the second mating of the P_1 animals: 19/25, 21/25, 19/25, 21/25 females from each group were pregnant, mean gestation length was 21.2, 21.8, 21.9, 21.6 d respectively and mean total number of pups delivered (F_{1b}) was 14.2, 13.6, 13.8, 13.1 per litter. There was no significant change in the number of pups that were stillborn, or missing/cannibalised. F_{1b} pup viability over the first 21 d post-partum was not affected (94 %, 93 %, 93 %, 95 % survival). The mean pup weight on the day of delivery was 6.0 g, 6.2 g, 6.2 g and 6.1 g and there were no significant effects on subsequent weight gain. As with the F_{1a} pups, there was no adverse effect on sex-ratio at delivery.

The profile of results from the mating of P_2 animals producing the F_{2a} and F_{2b} generations was similar to those noted above.

Organ weight data were obtained from P_1 and P_2 animals only, after about 27-30 weeks and 44-47 weeks of the study. In the P_1 generation, at 1200 ppm increased relative liver and thyroid weight was seen in males (17 % and 60 % greater than controls respectively) and females (12 % and 57 %). In addition, at this exposure level, relative kidney weight was increased in females (not recorded in P_1 males) by 14 % compared to controls and testes weight in males (9 %). These changes were replicated in the P_2 animals with increases of approximately the same magnitude.

There were no treatment-related macroscopic pathology abnormalities seen in P_1 animals. However, microscopically observable changes, consistent with other repeated-exposure

studies were seen in the thyroid, kidney and pituitary. Minimal to mild diffuse thyroid follicular hyperplasia was seen in all males and 22/25 females at 1200 ppm. Focal or nodular areas of thyroid follicular hyperplasia were seen in 2 males at 1200 ppm and one male at 120 ppm. In addition, thyroid follicular adenoma was seen in 3 males at 1200 ppm. Brown globular pigment was observed in the lumen of the proximal tubules of the kidneys amongst P_1 animals at 120 and 1200 ppm. However, in the absence of any observable alteration in kidney structure or function this finding is considered of doubtful toxicological importance. Minimal to moderate hypertrophy and/or vacuolation of individual cells in the adenohypophysis of the pituitary was observed in 10/24, 20/25, 18/25, and 20/25 males and 1/25, 9/24, 11/24, and 8/25 females of the 0, 30, 120, and 1200 ppm groups respectively. Again, these findings were replicated in the P_2 generation. Although investigations of parental animals are more limited than in a 'standard' repeated-exposure study, no adverse effects were observed in parental animals at around 8 mg $kg^{-1}d^{-1}$.

Overall, there were no adverse effects seen on fertility or pup development in this multi-generation study in which rats received up to approximately 80 mg kg^{-1} d^{-1} mancozeb before mating, and equating to up to around 180 mg kg^{-1} d^{-1} during lactation. These exposure levels produced evidence of parental systemic toxicity (such as reduced body weight gain, and histological changes in thyroid, liver and pituitary), indicating that the testing regime was rigorous.

3.3.7.2.2 Ethylene thiourea

Data presented in this Section are those reports provided by industry; no further published data were available. The only study provided on ETU by industry is a two-generation fertility study in rats:

In an unpublished two-generation study conducted in accordance with OECD guidelines, conforming to GLP, groups of 25 male and 25 female Sprague-Dawley rats received 0, 2.5, 25, or 125 ppm ETU (98 % pure) by dietary administration. Administration of ETU started at the age of 6 weeks and mating of the first generation (P_1) after 10 weeks of treatment. On day 4 post-partum, pups were culled to leave 4 males and 4 females per litter (the F_1 generation). Once the F_1 pups were weaned (day 21 post-partum), 25 males and 25 females were selected at random (to become P_2 animals) for mating after an 18-week treatment period, to produce F_2 pups. Excess F_1 pups were killed and examined macroscopically. Comprehensive macroscopic and microscopic pathology examinations were performed on P_1 and P_2 animals on completion of the 21-d lactation period and any animals found dead. Organ weight data were not obtained (Unpublished, 1990c).

The fixed dietary concentrations of ETU corresponded to approximately 0.1-0.4, 0.5-4, and 4-20 mg kg^{-1} d^{-1} for P_1 males and females. Exposure levels in terms of mg kg^{-1} d^{-1} during the lactation period were not provided. However, similar intakes were achieved by the P_2 generation.

There were no exposure-related mortalities and no clinical signs of toxicity amongst P_1 or P_2 animals. A slight reduction in body weight gain was noted amongst males receiving 125 ppm ETU; for P_1 males body weight gain was 7 % lower than controls during the 10-week pre-mating period. For P_2 males receiving 125 ppm body weight gain was 5 % lower than controls over the 18-week pre-mating period. P_1 and P_2 females were unaffected. Food consumption of males and females was not adversely affected.

The number of pregnant P_1 females was not adversely affected by ETU treatment (24-25/25 females from each group, including controls, were pregnant). Mean gestation length was similar between groups (21.5, 21.4, 21.4, 21.7 d). Post-implantation loss was calculated to be 13.3 %, 5.7 %, 10.3 %, and 12.6 %. The mean total number of pups delivered (F_1) was not adversely affected (13.3, 15.9, 14.2, 15.0 per litter) and there was no significant increase in the number that were stillborn, or dying during the lactation period. The mean pup weight on the day of delivery was 6.3g, 6.4g, 6.7g and 6.5g and there were no adverse effects on subsequent weight gain. On the day of delivery the sex-ratio of F_1 pups was not significantly altered (52 %, 46 %, 51 %, 55 % males).

The profile of results from the mating of P_2 animals producing the F_2 generation was similar to those noted above.

There were no treatment-related macroscopic pathology abnormalities seen in P_1 animals. However, microscopically observable changes were seen in the thyroid and pituitary. Thyroid follicular cell hypertrophy was seen in males and females (males, 1/25, 0/25, 0/25, 15/25; females, 0/25, 0/25, 3/25, 19/25) and hyperplasia noted (males, 1/25, 0/25, 5/25, 23/25; females, 0/25, 0/25, 1/25, 17/25). Anterior pituitary cell hypertrophy was seen in 16/25, 17/25, 21/25, and 24/25 males respectively and 0/25, 0/25, 0/25, and 20/25 females. Again, these findings were replicated in the P_2 generation. In addition, 3/25 thyroid follicular adenomas were reported at 125 ppm in P_2 males compared to none in other groups.

Overall, there were no adverse effects seen on fertility or pup development in this multi-generation study in which rats received up to approximately 20 mg kg^{-1} d^{-1} ETU before mating. These exposure levels produced evidence of parental systemic toxicity (such as a slight reduction in body weight gain, and histological changes in thyroid, and pituitary) indicating that the testing regime was rigorous. No adverse effects were observed in parental animals in this study at up to approximately 4 mg kg^{-1} d^{-1}.

3.3.7.3 SUMMARY OF REPRODUCTIVE EFFECTS

Only limited information on the developmental effects of zineb is available and such data are essentially restricted to effects in rats using the oral route. Marked developmental effects (micrognathia, phocomelia, encephalocoele and hydrocephaly) were seen in the pups of dams that received 2000 mg kg^{-1} orally during gestation. However, it was unclear whether or not there were also signs of maternal toxicity at this exposure level. No developmental effects were seen in rats at 1000 mg kg^{-1} in the same study. One inhalation study is available but due to limitations in reporting no useful conclusions can be drawn. Information from other species or routes of administration is lacking. More information is available on the structurally related mancozeb. Oral dosing with mancozeb to rats during gestation resulted in no effects on development at doses up to 60 mg kg^{-1} d^{-1}. An increase, compared to controls, in skeletal anomalies (reduced ossification of the intraparietal bone and increase in the size of the anterior fontanelle) were observed at 360 mg kg^{-1} d^{-1}, the highest dose used, a level associated with marked maternal toxicity. A brief report of a study in rabbits suggests no effects on development at 30 mg kg^{-1} d^{-1} with severe maternal toxicity occurring at 80 mg kg^{-1} d^{-1}, the next dose level tested. Mancozeb has also been tested via inhalation exposure with no effects seen on development at 55 mg m^{-3}, a level associated with maternal toxicity. An increase in skeletal anomalies was seen in fetuses at 110 mg m^{-3}. Overall, there is some evidence that mancozeb may induce an increase in skeletal anomalies, although only

at dose levels associated with maternal toxicity. ETU, the common metabolite of zineb and mancozeb is recognised as possessing the ability to affect development. In the rat, developmental effects (particularly hydrocephaly, and skeletal malformations) have been demonstrated in the absence of maternal toxicity following a single oral administration of 30 mg kg^{-1} at any time during days 13-20 of gestation, or repeated doses of 5 mg kg^{-1} during days 6-15. At higher exposures (around 200 mg kg^{-1}) mesenchymal necrosis of forelimb buds was noted, still in the absence of maternal toxicity.

There are no conventional studies that have specifically investigated effects on fertility. In the absence of high quality data on zineb, it is possible to read across information from the structurally related mancozeb and the potentially active metabolite ETU. Data only via the oral route of administration are available.

A high quality two-generation study using the oral gavage route indicates that there were no adverse effects on fertility even at doses that resulted in parental toxicity (about 80 mg kg^{-1} d^{-1} repeatedly). A similar study indicated that ETU itself did not result in impaired fertility at doses up to around 20 mg kg^{-1} d^{-1}. Hence, overall, although the fertility data on zineb are limited, the overall conclusion from the database including mancozeb and ETU is that such effects would not be anticipated at quantitatively similar doses.

3.3.8 HUMAN DATA, INCLUDING REPORTED EXPERIENCE OF MANUFACTURERS / APPROVAL HOLDERS

The information from studies in humans has been covered in detail under each toxicological endpoint. A brief summary of the available information is presented here, indicating those earlier Sections where more details are given. This is followed by reports of the experience of manufacturers and approval holders, submitted to HSE in response to the review.

Only a single case report was available relating to acute toxicity in a 42-year-old male who used a zineb-mancozeb mixture to spray cucumbers (see Section 3.3.1.1.3). Although toxic signs and symptoms were reported, probably due to his exposure, no firm conclusions can be drawn given a lack of objective information on exposure conditions. No skin irritancy was observed in 49/50 volunteers exposed to a '65 % zineb commercial wettable powder' (see Section 3.3.2.2). No information is available on eye or respiratory tract irritation in humans. With respect to skin sensitisation, a number of human case reports are available in which individuals have presented with (see Section 3.3.3.1.2) contact dermatitis and subsequently been found to respond to skin challenge with zineb. However, none of the cases provides conclusive evidence that zineb itself possess skin sensitisation potential. No reports of human sensitisation to zineb were found in either the EPIDERM (Occupational Skin Surveillance Survey, 1993 to 1999) or OPRA (Occupational Physicians Reporting Activity, 1994 to 1999) databases. No data are available for humans on the respiratory sensitisation potential of zineb.

No studies of workers repeatedly exposed only to zineb or mancozeb are available. In groups of flower bulb workers, slight differences were seen in measurements of nerve conduction velocities, however, the clinical significance of these findings is uncertain. It is not possible to draw any conclusions on the role zineb may have played (see Section 3.3.4.5).

In one study of workers exposed to a variety of pesticides, typically including mancozeb, a proportion of crop sprayers showed a slightly greater blood level of ETU compared to lesser

exposed farm owners and non-exposed controls (see Section 3.3.4.5). No effects on serum levels of TSH and T4 were detected in the potentially exposed workers. Supporting data are available from a study of workers involved in the production of mancozeb, where ETU-haemoglobin adducts were found in 40 % of workers. No other information on ETU-adducts following exposure to EBDCs is available. However, this finding is consistent with the observation from studies in animals of the metabolism of EBDCs to ETU.

Workers exposed to mancozeb and a variety of other chemicals including CS_2 showed a statistically significant increase in lymphocyte proliferative response and IL2 production (see Section 3.3.4.5). Due to inadequacies in exposure assessment in regard of other chemicals and previous exposures, and in light of the fact that no clinical signs of immunologically mediated disease was detected, the toxicological significance of this finding is unclear.

With respect to genotoxicity, cytogenetic analysis of the peripheral lymphocytes of workers potentially exposed to zineb and/or mancozeb have been carried out (see Section 3.3.5.2.3). However, due to limitations in the design and reporting of the studies no firm conclusions could be drawn from them. No studies were available in which the carcinogenic or reproductive toxicity potential of zineb in humans.

Three Approval holders responded to enquiries regarding the incidence of adverse human health effects of zineb (Unpublished, 1999a,b,c). No incidents have been reported in recent years. One was aware of a small number of reports of skin irritation in operators applying antifouling products containing zineb occurring approximately 20 years ago. However, these were attributed to the presence of TBTO (tributyltin oxide) in the antifouling products used.

The main manufacturer of zineb, reports that both technical zineb and products containing it have been manufactured in its Spanish plant since 1963 (Unpublished, 1999d). This plant has experienced no adverse effect associated with exposure to zineb. Between 6 and 15 workers are involved with the process.

No incidents associated with zineb- or mancozeb-containing antifouling products were reported to the Pesticide Incidents Appraisal Panel between 1989 and 1999.

The Belfast and Edinburgh Centres of the National Poisons Information Service have reported that they have received no enquiries concerning zineb in the periods from 1996 onwards and 1998 onwards, respectively (Unpublished, 1999e and f). The Cardiff Centre reported very brief details of 4 incidents related to antifouling products in the period 1997 - 1999 but was unable to relate these to specific products or active ingredients (Unpublished, 2000).

In addition, there have been no incidents related to zineb reported to HSE under RIDDOR (the Reporting of Injuries, diseases and Dangerous Occurrences Regulations) in the last 14 years.

3.4 DATA REQUIREMENTS

None have been identified

4 OPERATOR AND CONSUMER EXPOSURE AND RISK ASSESSMENTS

4.1 NO ADVERSE EFFECT LEVELS FOR USE IN RISK ASSESSMENTS

4.1.1 SUMMARY OF NOAELS

A summary of the no adverse effect levels for zineb and mancozeb, identified in the mammalian toxicity assessment, is set out in Table 4.1.

Table 4.1 NOAELs identified in the mammalian toxicity assessment

Acute Toxicity

Route	Substance	LD_{50} /LC_{50}	NOAEL/NOAEC
Oral	Zineb	7000 - 8000 mg kg^{-1}	Not identified
	Mancozeb	15000 mg kg^{-1}	9600
Dermal	Zineb	> 7000 mg kg^{-1}	> 7000 mg kg^{-1}
	Mancozeb	> 2000 mg kg^{-1}	> 2000 mg kg^{-1}
Inhalation	Mancozeb	> 4.76 mg l^{-1}	Not identified

Repeated exposure ≤ 90 d

Route	Substance	Species	Duration (d)	Basis for NOAEL/NOAEC (effects or site of action)	NOAEL/ NOAEC (mg kg^{-1} d^{-1} /mg.m^{-3})
Oral	Zineb	Rat	5	None	>0.05
	Zineb	Rat	28	Inhibition of liver MFO	5
	Mancozeb	Rat	90	Reduction in T4, resolved after 4 weeks recovery	7 - 8
	Mancozeb	Rat	90	Reduction in T4, Inhibition of liver MFO increased liver, thyroid and male spleen weight,	7
	Mancozeb	Rat	90	Long nerve lesions, loss of use of hind limbs apparently recoverable.	8 - 10
	Mancozeb	Dog	90	Thyroid/reproductive organs/haematology	3
Dermal	Mancozeb	Rat	28	None	1000
	Mancozeb	Rabbit	28	Body weight loss	250

Inhalation	Mancozeb	Rat	90	Reduction in T4, Body weight loss	40 $mg.m^{-3}$

Repeated exposure > 90 d

Route	Substance	Species	Dosing (weeks)	Basis for NOAEL (effects or site of action)	NOAEL ($mg\ kg^{-1}\ d^{-1}$)
Oral	Zineb	Dog	52	Thyroid	50
	Mancozeb	Dog	52	Haematological/liver/thyroid	7-8
	Mancozeb	Mouse	78	Body weight loss/ thyroid - reduced T4	13-18
	Mancozeb	Rat	104	Body weight loss/thyroid masses, follicular hyperplasia, reduced T4/ testes	4-7

Reproductive toxicity

Route	Substance	Endpoint	Species	Dosing Duration	NOAEL /NOAEC ($mg\ kg^{-1}\ d^{-1}$ /$mg.m^{-3}$)
Oral	Mancozeb	Fertility	Rat	-	> 80
	Zineb	Developmental Effects	Rat	Days 11, 13 and d 2-21 of gestation	1000 (skeletal malformations, increase late fetal death at 2000)
	Mancozeb	Developmental Effects	Rat	Days 6-15 of gestation	60 (maternal toxicity and skeletal anomalies at 360)
Inhalation	Mancozeb	Developmental Effects	Rat	Days 6-15 of gestation	55 $mg.m^{-3}$ (hind limb weakness, reduced body weight at 17; no developmental effects)

4.1.2 DISCUSSION OF NOAELS USED IN THIS RISK ASSESSMENT

When the mammalian toxicology of zineb was considered in 1999, the ACP agreed that the NOAEL of 3 $mg\ kg^{-1}\ d^{-1}$, derived from the 90 d study with mancozeb in the dog, should be used in the risk assessments for systemic toxicity to professional operators (including chandlers). The ACP agreed that this could be used as an equivalent NOAEL for zineb, in accordance with the discussions in Section 4. This NOAEL appeared also to be compatible with the available data on ETU toxicity, taking into account the approximate 20 % metabolism of zineb to ETU. The exposure of amateur users applying antifouling product was considered to be very short term and very infrequent and therefore comparison with endpoints for acute toxicity was considered appropriate.

Section 3.2.1.2.1 reports dermal penetration of less than 1 % for an aqueous suspension of zineb applied to rat skin. However, no information is available on the skin penetration of zineb (or mancozeb) when in solvent-based formulations such as antifouling products. For the purposes of risk assessment a value of 10 % penetration has been assumed. The skin

sensitization potential of zineb needs to be considered for both professional and amateur operators.

4.2 OPERATOR AND CONSUMER EXPOSURE

4.2.1 INFORMATION RELATING TO USER EXPOSURE

4.2.1.1 INDUSTRY DATA

Data were generated by an industry consortium in response to an earlier requirement for information on exposure to tributyltin. The report that was produced as a consequence of the exposure study, and which included a risk assessment, gave a suitable basis for risk assessment (Unpublished, 1995).

4.2.1.2 HSE DATA

Since 1994, the Health and Safety Executive has gathered information on human exposure to antifouling products in the professional and amateur sectors, to inform its role of assessing exposure and risk to operators and others. The information takes two forms:

- The pattern of work-the frequency and duration of potential exposure, the areas coated per session, the amount of product used and seasonal factors.

- The exposure-the median and realistic worst case exposures in applying the products and identified tasks or jobs.

Risk assessment patterns of use data are based on surveys and anecdote. They are as accurate as is possible but should not be regarded as definitive.

The surveys and studies informing HSE assessments are:

- 9 surveys applying copper-based antifouling products to ships (40 exposure data; Unpublished, 1994)
- 5 surveys applying tin-based antifouling products to ships (20 exposure data; Unpublished, 1996)
- 4 surveys applying various antifouling products to ships (10 exposure data; Unpublished, 1996)
- Pattern of use survey (2 commercial organisations, 4 service organisations for leisure craft; Unpublished, 2000)
- 8 surveys applying copper-based antifouling products to leisure craft (9 exposure data; Unpublished, 2000)

All HSE surveys took place in the north of England or Scotland. Some information on patterns of use was derived through exposure surveys. Each exposure data point comprised the potential dermal exposure (the amount of antifouling product depositing on the outer surface of the person), the exposure of hands inside protective or other gloves, exposure by inhalation, the tasks done and the amount of product used.

HSE holds no information on the relative market importance of products or active substances. Three-quarters of the products found being used in the 1994/5 survey were free association (conventional or contact leaching, i.e. the active ingredient leaches from the antifouling product) and one-quarter self-polishing (ablative, i.e. the active ingredient is bound in a copolymer antifouling product which hydrolyses slowly in sea water). HSE has no reliable data relating to exposures in the military sector, where data relate only to airborne concentrations of copper. There are no data concerning other immersed structures (e.g. oil-rigs, jetties, fish-farm installations), nor on exposure in stripping expired antifoulings.

4.2.1.3 ENVIRONMENT AGENCY DATA

The Environment Agency (EA) (1998) commissioned a report on environmental problems from antifouling agents that contained information on patterns of use (Boxall *et al*., 1998).

4.2.1.4 EXPRESSION OF EXPOSURE DATA

All HSE data are quoted in terms of the antifouling product being applied and are time-weighted. Data are therefore normalised and in the forms 'mg product h^{-1}' for dermal exposure and 'mg product m^{-3}' for inhalation exposure, respectively (Unpublished, 1998). The sampling methods for potential dermal exposure using patches have been validated for spraying activities (Unpublished, 1996 and Glass *et al*., 2002*) (*Unpublished at the time of the review, published subsequently – see references). However, they have not been validated for application by brush and roller or paint handling.

It is inappropriate to express 'exposure' simply as a single value. In fact, there are exposure distributions. However, data are sparse due to the difficulty and high cost of their acquisition, and the exact nature of the distribution cannot be proven. Consequently, complex statistical treatments are considered inappropriate. HSE statisticians gave their opinion that in cases where data are sparse, it is valid to consider in detail only the non-zero results. Results with an effective value of zero can be taken into account in the 'frequency' or chance that exposure will occur.

The median value in a distribution, moderated by this frequency, represents a 'central tendency' value. The 95^{th} percentile data point of non-zero values represents a realistic worst case. If the distribution data are sparse, the highest values found may be taken as the worst case. Where the highest data point is a clear outlier (i.e. many times higher than the next highest point), a decision can be taken to note that datum but disregard it for the purposes of risk assessment.

4.2.1.5 MITIGATION OF EXPOSURE TO ANTIFOULANTS

Work clothing, whether or nor constituting formal personal protective equipment, does have protective properties. Impermeable clothing (e.g. Tyvek suit) will stop liquids reaching the skin, but easily becomes contaminated inside and is difficult to clean properly. Furthermore, deposits may concentrate on the equipment surface and are available for dislodging. Cloth-based equipment will tend to retain liquids and dusts to some extent; there will also be run-off. Penetration of clothing occurs through liquids soaking through seams, zips and elasticated parts, through being rubbed through by frequent contact and by being drawn through openings at neck, wrist and ankle by the 'bellows effect' when the operator moves inside the clothing. HSE data indicate a median 4 % penetration of the outer layer of work

clothing, generally a coverall. Having more than one layer of work clothing will provide better protection.

Protective gloves are generally effective in protecting the hands from antifouling product. However, hand contamination is inevitable, and the exposure route may be through putting on and taking off used gloves. One measure to mitigate continuing exposure through protective gloves being contaminated inside is for regular replacement of protective gloves, for example following each antifouling job.

Respiratory protective equipment (RPE) needs to achieve two objectives: to mitigate exposure by inhalation and to protect the skin of the face, head and neck. In general, a high standard of respiratory protective equipment with a workplace protection factor of at least 50 is needed for sprayers and possibly other RPE for some ancillary workers. The person at greatest risk is the sprayer, and for spraying, such protective equipment should be mandatory. If the pot-men were to work in the vicinity of the spray plume, then COSHH is likely to indicate that they should wear appropriate protective equipment. Appropriate equipment would probably be of a standard equivalent to an FFP3 disposable filtering facepiece respirator or better. However, the need for respiratory protection for workers other than sprayers is considered to be a matter for the COSHH workplace risk assessment. It is considered unlikely that amateur users would be able adequately to select and use RPE.

4.2.1.6 FACTORS AFFECTING EXPOSURE ESTIMATES FOR THE USE OF ANTIFOULING PRODUCTS

When the partial reviews of the physical chemistry and mammalian toxicology of the booster biocides were presented to the ACP in 1999, the ACP agreed that the assessment of risk to users and bystanders should be revised to take into account all factors affecting exposure and increase the clarity and transparency of the risk assessments. The TERs of potential concern related to the high-end exposures (95^{th} percentile or higher) that were calculated from the operator exposure model. For antifouling applications, the high-end predictor is based on the 95^{th} percentile for sprayers and the worst-case results for pot-men.

4.2.1.6.1 Dermal exposure

HSE believes the antifouling model to be an accurate predictor of the amount of product that deposits on the outer surfaces of a worker's clothing- as a worst case this amounts to in excess of 120 g of product in a spray session. The amount available for uptake following transfer to the skin is another matter. The estimation of systemic dose, resulting from conditions that can lead to 120 g of paint on the outside of clothing is open to interpretation and requires a degree of professional judgement to reach a realistic conclusion. In addition, the dermal absorption value used in the risk assessment is also critical to the resulting estimate of systemic dose.

4.2.1.6.2 Extent of penetration through clothing

HSE data indicate that 4 % is a realistic figure to adopt for the penetration of antifouling products through a single layer of typical protective workwear. Information from a number of sources suggests that the performance of protective clothing may be related to the level of the challenge. Laboratory experiments indicate that the higher the challenge, the lower the proportion of product that will penetrate to become available as a potential source for contact with the skin. This phenomenon has also been observed during field studies, such as those

reported by the Institute of Occupational Medicine (Unpublished, 1994) during the investigation of sheep dipping practices and field-effectiveness of PPE. Further layers of clothing will provide an extra barrier to skin contact.

Custom within the antifouling industry, brought about by a recognition that formulations may be unpleasant to work with and are difficult to remove from the skin, is for operators to protect themselves well, and often to wear two sets of coveralls. HSE is prepared to accept that it may be possible, at the higher levels of contamination, to consider a penetration factor through coveralls and clothing, and then onto the skin, at about 1 %. The value for penetration at 1 % is seen as the practical lower limit for modelling purposes as there will always be the potential for contact of product with exposed skin (e.g. around wrists, face and neck and through handling previously contaminated clothing). The ability of protective clothing to reduce potential exposures by at least two orders of magnitude has been demonstrated in the studies by the IOM (Unpublished, 1996) and is supported by the experimental findings related to penetration compared to challenge which are built into the POEM model (Anonymous, 1992).

4.2.1.6.3 Extrapolation from clothing to skin to systemic dose

The modelling process is not very good at estimating how much of the product finds its way to the skin, and how much of the active substance in the product is eventually absorbed. The current HSE estimates are precautionary and based on all of the product that is predicted to penetrate the layers of clothing getting to the skin, and a quantity of the active substance within the product immediately penetrating through to become a systemic dose. The amount of uptake via the skin may be established through dermal absorption studies, or if not available by using a default value such as 10 %. However, such uptake is considered unlikely to happen in reality for a number of reasons:

- Only a proportion of what lands on the skin is available to be absorbed when product deposition occurs in the form of spots or blobs, as active substance will be contained within the matrix of the dried-on product.

- The model does not take into account the dynamics of deposition or absorption, or the kinetics of metabolism.

- The model does not take into account actions to remove residues after work, particularly from the hands.

4.2.1.6.4 Patterns of exposure

A further factor relates to interpretation of the findings on exposure. Account needs to be taken of the pattern of work for a painter and the pattern of use of any particular product. Professionals spend much of their time carrying out preparatory work during vessel refitting-many other jobs take place while a vessel is in dry dock and it may be there for a number of weeks. Consequently, exposure to antifouling products is irregular with long intervals between exposures. Exposure to one particular active substance, other than copper compounds, is even less frequent. The most realistic worst case exposure scenario is that a painter may be exposed for no more than two or three days a month, but not every month, and then not to the same active substance. High-end exposures do not occur every time and could be considered as acute.

4.2.1.6.5 Hand exposure

Estimates of hand contamination play an important part in development of the exposure assessment for antifoulants. This is particularly true for the pot-men who come into contact with large amounts of product while of replenishing reservoirs for sprayers. There is always the opportunity for spillage. Where pot-men have worn suitable and adequate new gloves, it appears the levels of contamination to the hands have generally been low. Where pot-men have worn inappropriate gloves, old gloves, or no gloves at all, elevated exposures have been registered. These elevated exposures heavily influence the model. Appropriate changing of gloves suggests that hand exposure can be reduced.

For the sprayer, hand exposures tend to be lower than for the pot-man and are not a main driver of the exposure estimate.

4.2.1.6.6 Inhalation Exposure

Sprayers will always need to wear respiratory protective equipment. For the pot-men, exposure to spray aerosol is intermittent and unusual. Results indicate that the normal range (18 of 19 results) is between 0.2 and 4.0 mg m^{-3} of product. One exceptional result (the highest recorded) has been recalculated at 24 mg m^{-3} (previously 42 mg m^{-3}, but closer inspection of the proportion of active material in the product has caused abatement of this particular result). However, it is considered to be unlikely that even a result of this magnitude would be a true reflection of the personal exposure of a pot-man to aerosol. The individual result showed no elevated potential dermal exposure. HSE has judged that, when considered in the context of the other samples within the data set, the specific result of 24 mg m^{-3} did not reflect exposure to inhalable aerosol; the result was more likely to have arisen through direct transfer of antifoulant and possibly by direct contamination.

4.2.2 EXPOSURE ASSESSMENT-ANTIFOULANT SPRAYING

4.2.2.1 INTRODUCTION

Industry data are taken from the study ‘Determination of exposure to tin during commercial application of antifouling paint to ship hulls’ (Unpublished, 1995).

The following data are taken from 'Dermal exposure to non-agricultural pesticides', prepared by the HSE (Anonymous, 2000), taking account of the Environment Agency's 1998 technical report (Boxall *et al*., 1998). For roller and brush painting, data presented in Garrod *et al* (2000) have been assumed to apply to professionals.

4.2.2.2 PATTERNS OF USE IN SPRAY OPERATIONS-PROFESSIONAL USERS

Industry information on patterns of use accords reasonably well with existing knowledge and reflects current practice.

The work involves dry-docking for vessels the size of tugs and above, or docking on a hard surface for small fishing vessels. During the time for removing and re-applying antifouling product, general overhaul and refitting takes place. Consequently, applying the antifouling product is a minor proportion of the time spent working on the vessel. Workers remove and apply surface coatings over the ship, (e.g. bilges, holds), using two-pack epoxy preparations

for example. The pattern-of-use survey indicated that up to 10 % of employees' time might be spent in working with antifoulings.

Professionals work year-round. The vessel is cleaned with a high-pressure water-jet (for self-polishing coatings) or with abrasive grit (for erodible coatings). Bare metal surfaces are prepared with coatings such as corrosion inhibitors. The antifouling product is then applied using airless spray techniques at up to 100 bar. Sufficient sprayers are employed to ensure that one coat is applied in one workday. Rarely are more than two coats applied.

Applying antifouling product requires 2 to 4 persons per spray position. The three identified tasks are:

- Spraying - the sprayer
- Mixing and loading - the pot-man (who prepares the antifouling product and ensures Supply to the high pressure pump)
- Ancillary - the rein or tender men, who attend to keeping paint lines free and may also manoeuvre the mobile access platform (cherry-picker)

Antifoulings are applied on several days a month, for no more than two consecutive days a week. There would normally be one coating session per day. In the HSE surveys the duration of daily work ranged from 40 to 360 min per coating session (median 184 min), for each of the three identified tasks.

There are only estimated data for the quantity of antifouling product used per spray session. The quantity used ranged from 25 to over 800 l of antifouling product (median 240 l). The vessel surface areas coated ranged between 600 and 4000 m^2 (median 1600 m^2).

Where safety data sheets are supplied with products to professional end-users, this supply is often through the ship owner, who does not necessarily transmit the data sheet. Contractors rely on a compendium of data sheets that may become outdated. Contractors were found to have a default set of equipment, risk assessments and personal protective equipment that they used for most situations. In all surveys, overalls and gloves were found to be available for use, although these items were not always 'suitable'. Respiratory protective equipment was always found to be available. Sprayers usually took steps to protect any exposed skin from antifoulants.

4.2.2.3 EXPOSURE DATA FOR SPRAY OPERATIONS - PROFESSIONAL USERS

Detailed calculations are presented in Appendix 2, Tables 2.1 to 2.7. These are based on a 3 h shift and (as outlined in Section 4.2.1.5), a value of 4 % for penetration of a single layer of work clothing and a value of 1 % for penetration of a double layer of work clothing, for sprayers, pot-men and other professional operators such as tenders. Exposure data are quoted as in-use concentration of antifouling product on the skin and inhaled.

Industry data were presented in terms of the tin content of the product applied (3.25 % w/w). The amounts of tin measured were extrapolated back to an estimate of product exposure. The Industry and HSE estimates of potential dermal exposure may be compared (mg product h^{-1}):

Operator	Industry		HSE	
	Central tendency	Maximum	Central tendency	Maximum
Sprayer	10500	49800	6170	44700
Pot-man	1950	6550	2940	15000

The data are sufficiently similar to give confidence that the industry study and HSE survey data overlap, despite the different sampling media used to measure potential dermal exposure. This suggests that the industry study and the HSE surveys belong to the same distribution. Consequently, no separate risk assessment will be conducted on the industry data.

4.2.2.4 PATTERNS OF USE IN SPRAY OPERATIONS - AMATEUR USERS

There is no information that amateurs use spraying to apply antifouling, though this mode of application may be permitted in the conditions of approval. Clearly, spraying of leisure craft would be of shorter duration than the median around 3 h, which is typical for professionals. In contrast with application by brush and roller, (Section 4.2.5.2.2), there is often a clear need for respiratory and skin protection in spray application of antifoulants and it is unlikely that amateurs would be able to select or use personal protective equipment adequately. This suggests that approval of application of antifoulants by spraying by amateurs may be inappropriate. The ACP's views were sought on this issue.

4.2.2.5 EXPOSURE DATA FOR SPRAY OPERATIONS -AMATEUR USERS

While there are no directly related exposure data, the data model relevant to professional sprayers should apply, assuming no RPE. Amateur sprayers are assumed to wear a coverall and therefore clothing penetration of 4 % has been used in calculations (unlike amateur use of brush and roller in Section 4.2.4.2).

4.2.3 SYSTEMIC EXPOSURES IN SPRAY OPERATIONS

4.2.3.1 CALCULATIONS

The data sets and exposure calculations are set out in Appendix 2, Tables 2.1 - 2.7.

Assumptions:

- 20 % active substance in the product.
- 10 % dermal penetration default.
- 4 % or 1 % clothing penetration.

Exposure estimates for all professional workers are summarised in Tables 4.2 - 4.7. Calculations are presented for clothing penetration at 4 % and at 1 %, for standard workwear and additional PPE respectively as explained in Section 4.2.1.6. The calculation for professional sprayers includes respiratory protection by air-fed RPE with a 50-fold protection factor. For amateur spraying, calculations are presented in Table 4.8, with 4 % clothing penetration as explained in Section 4.2.2.4.

Table 4.2 Contact and systemic exposure to zineb for professional sprayers: 4% clothing penetration

Exposure item	Central Tendency	Worst Case
Amount of product in contact with skin (mg d^{-1}) *[3 h job, 4 % clothing penetration, 6170 mg h^{-1} central tendency, 44700 mg h^{-1} 95th percentile, 60 (median) & 241 (worst case) mg h^{-1} of product in-glove]*	920	6080
Percentage of active substance in product (%)	20	20
Amount of zineb in contact with skin (mg d^{-1})	184	1216
Dermal absorption value (%)	10	10
Exposure to zineb via dermal route (mg d^{-1})	18	122
Intake of product by inhalation **with RPE** (mg d^{-1}) *[3 h job, 1.25 m^3 h^{-1} inhaled volume, central tendency 6 mg m^{-3}, worst case 64.6 mg m^{-3} of product]*	0.45	4.84
Amount of zineb inhaled (mg d^{-1})	0.1	1
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1}) wearing RPE	0.3	2

Table 4.3 Contact and systemic exposure to zineb for professional pot-men: 4% clothing penetration

Exposure item	Central Tendency	Worst Case
Amount of product in contact with skin (mg d^{-1}) *[3 h job, 4 % clothing penetration, 2940 mg h^{-1} central tendency, 15000 mg h^{-1} 95th percentile, 35 (median) & 1380 (worst case) mg h^{-1} of product in-glove]*	458	5940
Percentage of active substance in product (%)	20	20
Amount of zineb in contact with skin (mg d^{-1})	92	1188
Dermal absorption value (%)	10	10
Exposure to zineb via dermal route (mg d^{-1})	9	119
Intake of product by inhalation (mg d^{-1}) *, without RPE. *[3 h job, 1.25 m^3 h^{-1} inhaled volume,, central tendency 0.6 mg m^{-3}, realistic worst case 3.84 mg m^{-3} of product]*	2.25	14.4
Amount of zineb inhaled (mg d^{-1}) (ii)	0.5	3
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1})	0.2	2

* Excludes top data point that is considered an outlier. This operator had significantly lower dermal exposure (by a factor of between 10 and 30) than each of the others in the study and his inhalation exposure was higher than that recorded for his associated sprayer-this would not be expected and cannot be explained. HSE concluded the sample has become contaminated and, consequently, the point discarded.

Table 4.4 Contact and systemic exposure to zineb for other professional operators: 4% clothing penetration

Exposure item	Central Tendency	Worst Case
Amount of product in contact with skin (mg d^{-1}) *[3 h job, 4 % clothing penetration, 885 mg h^{-1} central tendency, 3470 mg h^{-1} 95th percentile, 35 (median) & 180 (worst case) mg h^{-1} of product in-glove*	211	956
Percentage of active substance in product (%)	20	20
Amount of zineb in contact with skin (mg d^{-1})	42	191
Dermal absorption value (%)	10	10
Exposure to zineb via dermal route (mg d^{-1})	4	19
Intake of product by inhalation (mg d^{-1}) *[3 h job, 1.25 m^3 h^{-1} inhaled volume, central tendency 0.8 mg m^{-3}, realistic worst case 4.8 mg m^{-3} of product]*	3	18
Amount of zineb inhaled (mg d^{-1})	0.6	4
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1})	0.1	0.4

Table 4.5 Contact and systemic exposure to zineb for professional sprayers: 1% clothing penetration

Exposure item	Central Tendency	Worst Case
Amount of product in contact with skin (mg d^{-1}) *[3 h job, 1 % clothing penetration, 6170 mg h^{-1} central tendency, 44700 mg h^{-1} 95th percentile, 60 (median) & 241 (worst case) mg h^{-1} of product in-glove]*	365	2060
Percentage of active substance in product (%)	20	20
Amount of zineb in contact with skin (mg d^{-1})	73	412
Dermal absorption value (%)	10	10
Exposure to zineb via dermal route (mg d^{-1})	7	41
Intake of product by inhalation **with RPE** (mg d^{-1}) *[3 h job, 1.25 m^3 h^{-1} inhaled volume, central tendency 6 mg m^{-3}, worst case 64.6 mg m^{-3}, of product]*	0.45	4.84
Amount of zineb inhaled (mg d^{-1})	0.1	1
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1}) wearing RPE	0.1	0.7

Table 4.6 Contact and systemic exposure to zineb for professional pot-men: 1% clothing penetration

Exposure item	Central Tendency	Worst Case
Amount of product in contact with skin (mg d^{-1}) *[3 h job, 1 % clothing penetration, 2940 mg h^{-1} central tendency, 15000 mg h^{-1} 95th percentile, 35 (median) & 1380 (worst case) mg h^{-1} of product in-glove]*	193	4590
Percentage of active substance in product (%)	20	20
Amount of zineb in contact with skin (mg d^{-1})	39	916
Dermal absorption value (%)	10	10
Exposure to zineb via dermal route (mg d^{-1})	4	91.6
Intake of product by inhalation (mg d^{-1}) *, without RPE. *[3 h job, 1.25 m^3 h^{-1} inhaled volume, central tendency 0.6 mg m^{-3}, realistic worst case 3.84 mg m^{-3} of product]*	2.25	14.4
Amount of zineb inhaled (mg d^{-1})	0.5	3
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1})	0.1	1.6

* Excludes top data point that is considered to be an outlier. This operator had significantly lower dermal exposure (by a factor of between 10 and 30) than each of the others in the study and his inhalation exposure was higher than that recorded for his associated sprayer-this would not be expected and cannot be explained. HSE concluded the sample has become contaminated and, consequently, the point discarded.

Table 4.7 Contact and systemic exposure to zineb for other professional operators: 1% clothing penetration

Exposure item	Central Tendency	Worst Case
Amount of product in contact with skin (mg d^{-1}) *[3 h job, 1 % clothing penetration, 885 mg h^{-1} central tendency, 3470 mg h^{-1} 95th percentile, 35 (median) & 180 (worst case) mg h^{-1} of product in-glove]*	131	644
Percentage of active substance in product (%)	20	20
Amount of zineb in contact with skin (mg d^{-1})	26	129
Dermal absorption value (%)	10	10
Exposure to zineb via dermal route (mg d^{-1})	3	13
Intake of product by inhalation (mg d^{-1}) *[3 h job, 1.25 m^3 h^{-1} inhaled volume, central tendency 0.8 mg m^{-3} realistic worst case 4.8 mg m^{-3} of product)*	3	18
Amount of zineb inhaled (mg d^{-1})	0.6	4
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1})	0.1	0.3

Table 4.8 Contact and systemic exposure to zineb for amateur sprayers: 4% clothing penetration

Exposure item	Central Tendency	Worst Case
Amount of product in contact with skin (mg d^{-1}) *[1 h job, 4 % clothing penetration, 6170 mg h^{-1} central tendency, 44700 mg h^{-1} 95th percentile, 60 (median) & 241 (worst case) mg h^{-1} of product in-glove]*	306	2030
Percentage of active substance in product (%)	20	20
Amount of zineb in contact with skin (mg d^{-1})	61	406
Dermal absorption value (%)	10	10
Exposure to zineb via dermal route (mg d^{-1})	6	41
Intake of product by inhalation **without RPE** (mg d^{-1}) *[1 h job, 1.25 m^3 h^{-1} inhaled volume, central tendency 6 mg m^{-3}, worst case 64.6 mg m^{-3} of product]*	7.5	80.7
Amount of zineb inhaled (mg d^{-1})	2	16
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1})	0.1	1

4.2.3.2 ASSESSMENT OF RISKS DURING SPRAYING

The levels of exposure estimated for professional and amateur workers have been used to calculate the toxicity: exposure ratios (TERs) set out in Table 4.9.

Table 4.9 Summaries of Tables 4.2 To 4.8 for spraying operations

User	Central tendency exposure (mg kg^{-1} d^{-1})	TER	Worst case exposure (mg kg^{-1} d^{-1})	TER
Professional users (NOAEL 3 mg kg^{-1} d^{-1})				
4 % clothing penetration				
Sprayer	0.3	10	2	2
Pot-man	0.2	15	2	2
Ancillary worker	0.1	30	0.4	8
1 % clothing penetration				
Sprayer	0.1	30	0.7	4
Pot-man	0.1	30	1.6	2
Ancillary worker	0.1	30	0.3	10
Amateur users (LD_{50} 7000 mg kg^{-1})				
Amateur sprayer	0.1	70,000	1	7,000

4.2.3.2.1 Professionals

The current risk assessment highlights a potential cause for concern. However, exposure calculations were based on an estimated 10 % skin penetration of zineb from antifouling products. In addition, the ACP considered that the known greater tolerance of humans to thyroid stimulation (stimulation in the human thyroid, e.g. in iodine deficiency, does not

normally lead to tumours) suggested that the NOAEL selected could be viewed as conservative.

In 1999, when the ACP considered the risk assessment, they agreed that approval holders should be required to carry out a study of skin penetration in order to enable the exposure estimates to be refined. In the interim it was agreed that further personal protective equipment (PPE), including respiratory protective equipment (RPE) be specified to mitigate the estimated exposures. Fuller details are given in Appendix 1. The ACP agreed that all professional operators exposed to antifouling products containing zineb should wear a disposable coverall with hood (providing head protection) and a second overall beneath this coverall of a contrasting colour to the antifouling product being applied. All bare skin was to be covered and the disposable coverall used for no more than one spraying session. The overall beneath was to be changed regularly and whenever product break-through had been detected. In addition, gloves were to be changed after each spraying operation. Professional sprayers were required also to wear RPE, including air-fed respiratory equipment with combined protective helmet and visor. These PPE specifications were considered also to mitigate the risks of skin sensitization and respiratory irritation. For non-sprayers, the need for RPE was to be informed by a COSHH assessment.

The higher TER values calculated using a figure of 1 % penetration of clothing to take account of such PPE, confirm that the requirements are necessary. In addition to the PPE specified previously, the ACP agreed that professional users should be required also to wear impervious footwear that protects the lower leg. This is good practice and is consistent with the requirements for other active ingredients used in antifoulings.

4.2.3.2.2 Amateurs

While amateur products have been approved for spraying, there is no information to indicate that this happens. The TERs calculated indicate that short-term exposure to zineb would not give rise to concerns for systemic toxicity. However, the partial review in 1999 identified that there might be concerns due to the potential for skin sensitization and possibly for respiratory irritation. At that time, the ACP considered that zineb might be considered a weak skin sensitiser but that the data on human experience did not indicate any grounds for serious concern. No data provided to this review suggested concerns for respiratory irritation. Therefore spray application of zineb-containing antifouling products was permitted to continue. However, as the potential for dermal exposure during spraying is much greater than during application by brush or roller, the ACP concluded that approvals for spraying by amateur users should be revoked.

4.2.4 EXPOSURE ASSESSMENT-APPLICATION OF ANTIFOULING PRODUCT BY BRUSH AND ROLLER

The following data are taken from 'Dermal exposure to non-agricultural pesticides', prepared by the HSE (Anonymous, 2000). No data have been received from industry and there are no data directly related to professional users.

4.2.4.1 PATTERNS OF YSE IN BRUSH AND ROLLER APPLICATION - AMATEUR AND PROFESSIONAL (CHANDLERS) USERS

The HSE’s information is that the work is seasonal (springtime), and may be performed on a slipway, hard-standing or chandler's yard. Boat owners comprise the great majority of users,

with a very small proportion of boats being treated with antifouling product by chandlers. The boat is cleaned with a high-pressure water-jet and may be scraped. The antifouling product is applied using paintbrush or paint roller. Rarely are more than two coats applied. Applying antifouling product employs no more than 2 persons per vessel. While amateur products have been approved for spraying, there is no information to indicate that this happens. Nor is there any information on the use of aerosol spray packaged antifouling products for spot usage. The quantity of antifouling product used per application session was found to range between 1.5 and 5 l (median 4 l; these were all copper-based products). Normally, 2 coats were applied to boat surfaces that were found to range between 14 and 30 m^2 (median 20 m^2). Between 0.09 and 0.27 l were applied per square metre (median 0.22 l m^{-2}) at a work rate between 2.3 and 7.5 min m^{-2} (median 4.4 min m^{-2}).

The EA report has some comparative data on active substance and product usage. It indicated that the average boat size was around 30 ft (approximately 9 m), with motorboats (about 25 % of all boats) generally larger than sailing boats (about 75 % of all boats). Fouling was removed by pressure washing and exhausted antifouling product removed with abrasive and/or a stripping preparation. Most boat owners applied antifouling product themselves using a paint roller, annually. Around 15 % of owners applied antifouling product less than annually. The quantities applied ranged from 0.1 to 0.3 l per square foot (0.09 m^2) of boat. Application of antifouling product took place mostly on hard standing or in a boat park. Chandlers were found generally to sell more than 23 products: 163 chandlers belonged to the British Marine Industries Federation, accounting for about 70 % of all chandlers. Antifouling product was sold mostly between February and May.

Amateurs normally apply antifouling products over one day or two consecutive days, normally during fine weather. The application time was found in the HSE survey to range between 35 and 112 min (median 90 min).

4.2.4.2 WORK CLOTHING

Work clothing worn by amateurs provides some degree of protection. While clothing will tend to retain liquids and dusts to some extent, there will also be run-off. Amateurs were found normally to wear coveralls and cloth gloves for application by brush and roller. However, anecdotal evidence suggests that antifouling product application wearing minimal clothing is not uncommon. It is expected that chandlers would wear work clothing and protective gloves.

Certain clothing may be desirable to mitigate general risks to health and safety, such as skin contact with solvent-based products. In such cases, gloves may be specified as a precautionary measure for amateurs to ensure that such general risks are minimised. In addition, certain risk phrases derived through the CHIP (Chemicals (Hazard Information and Packaging for Supply) Regulations) process carry mandatory safety phrases and these will appear on a label even though there may be negligible risk to amateurs using the product.

While amateurs may wear clothing such as coveralls or a long sleeved shirt and long trousers to apply non-agricultural pesticides, this cannot be assured, so may not be assumed for risk assessment purposes. Where there is no better information, default values for penetration to the skin are proposed at 5 % when wearing clothes that provide some degree of protection (professionals at all times) and at 50 % for the realistic worst cases i.e. when minimal

clothing is worn (by amateurs). The realistic worst case for amateurs also assumes that no gloves are worn.

4.2.4.3 EXPOSURE DATA FOR BRUSH AND ROLLER APPLICATION

Detailed calculations are presented in Appendix 2, Tables 2.8 to 2.10. The very sparse exposure data relate to 9 amateurs painting their own vessels, with the boat on a cradle, trailer or sling. All but one of the jobs were outdoors, with both brush and roller used in most cases. There were 9 data for potential dermal exposure, 7 data for exposure inside gloves and 2 data for exposure to bare hands. Of the 9 subjects, only 4 showed any exposure by inhalation. About half of the potential dermal exposure was to the legs.

4.2.5 SYSTEMIC EXPOSURES IN BRUSH AND ROLLER APPLICATION

4.2.5.1 CALCULATIONS

The data sets and exposure calculations are set out in Appendix 2, Tables 2.8 to 2.10.

Assumptions:

- 20 % active substance in the product.
- 10 % dermal penetration default.
- 5 % or 50 % (amateur worst case) clothing penetration.

For professional application of antifouling products by brush and roller, calculations are presented in Table 4.10 with 5 % clothing penetration for workwear as explained in 4.2.4.2. Calculations for amateurs are summarised in Table 4.11 and are presented for clothing penetration at 5 % and 50 %, as also explained in Section 4.2.4.2.

Table 4.10 Contact and systemic exposure to zineb for professionals (chandlers): application by brush and roller

Exposure item	Central Tendency	Worst Case
Amount of product in contact with skin (mg d^{-1}) *[1.5 h job; 5 % clothing penetration; 1020 mg h^{-1} central tendency, 6480 mg h^{-1} 95th percentile; 31 (median) & 1110 (worst case) mg h^{-1} of product in-glove]*	123	2160
Percentage of active substance in product (%)	20	20
Amount of zineb in contact with skin (mg d^{-1})	25	432
Dermal absorption value (%)	10	10
Exposure to zineb via dermal route (mg d^{-1})	3	43
Intake of product by inhalation (mg d^{-1}) *[1.5 h job, 1.25 m^3 h^{-1} inhaled volume, central tendency 0.02 mg m^{-3}, realistic worst case 0.11 mg m^{-3}, of product]*	0.04	0.21
Amount of zineb inhaled (mg d^{-1})	0.01	0.04
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1})	0.05	0.7

Table 4.11 Contact and systemic exposure to zineb for amateurs: application by brush and roller

Exposure item	Central Tendency	Worst Case
Amount of product in contact with skin (mg d^{-1}) *[1.5 h job; 5 % central tendency clothing penetration, 50 % worst case - minimal clothing; 1020 mg h^{-1} central tendency, 6480 mg h^{-1} 95th percentile; 31 (median) & 4400 (worst case - no gloves) mg h^{-1} of product on hand]*	123	11500
Percentage of active substance in product (%)	20	20
Amount of zineb in contact with skin (mg d^{-1})	25	2300
Dermal absorption value (%)	10	10
Exposure to zineb via dermal route (mg d^{-1})	3	230
Intake of product by inhalation (mg d^{-1}) *[1.5 h job, 1.25 m^3 h^{-1} inhaled volume, central tendency 0.02 mg m^{-3}, realistic worst case 0.11 mg m^{-3} product]*	0.04	0.21
Amount of zineb inhaled (mg d^{-1})	0.01	0.04
Total systemic exposure for 60 kg person (mg kg^{-1} d^{-1})	0.05	4

4.2.5.2 ASSESSMENT OF RISKS DURING APPLICATION BY BRUSH AND ROLLER

The levels of exposure estimated for professional and amateur workers have been used to calculate the toxicity:exposure ratios (TERs) set out in Table 4.12.

Table 4.12 Summaries of Tables 4.10 and 4.11 for application by brush and roller

User	Central Tendency Exposure (mg kg^{-1} d^{-1})	TER	Worst Case Exposure (mg kg^{-1} d^{-1})	TER
Professional users (NOAEL 3 mg kg^{-1} d^{-1})				
5 % clothing penetration				
Chandler	0.05	60	0.7	4
Amateur users (LD_{50} 7000 mg kg^{-1})				
5 % and 50 % clothing penetration				
Amateur	0.05	140,000	4	1,750

4.2.5.2.1 Professionals (chandlers)

Systemic exposure of chandlers is considered acceptable at the central tendency but gives cause for concern at the worst-case level. As explained in Section 4.2.3.2 for spray operations, the ACP has previously agreed that a better estimate of skin penetration of zineb from antifoulants would enable the risk assessment to be refined. In the interim, it was agreed that chandlers applying zineb-containing antifoulings by brush and roller should be required to wear a standard of personal protective equipment equivalent to that used by other professionals. The details of this are set out in Appendix 1. However, the potential for intake of zineb by inhalation during application by roller or brush is considered to be low and the RPE is not considered necessary.

4.2.5.2.2 Amateurs

Systemic exposure of amateurs is considered acceptable. The potential for intake of zineb by inhalation during application by roller or brush is considered to be low and the risk is considered acceptable. However, there will be dermal exposure, whether or not amateurs wear workwear. As explained in Section 4.2.3.2.2, when the partial review of zineb was considered in 1999, the ACP considered that zineb might be considered a weak skin sensitiser but that the data on human experience did not indicate any grounds for serious concern. No data provided to this review suggested concerns for respiratory irritation. Therefore application of zineb-containing antifouling products by brush and roller was permitted to continue. HSE considers it appropriate to continue approval for application by brush and roller, with a recommendation for gloves to be worn as a precautionary measure. This is compatible with requirements triggered by the CHIP classification. The ACP considered that this was acceptable for application by amateurs using brush and roller. It should be noted that information provided recently by 2 of the 3 companies that hold approval for amateur products suggests that little, if any, antifouling product containing zineb, is currently supplied to the amateur market.

4.2.6 EXPOSURE AND RISK ASSESSMENT FOR BYSTANDERS

No data are available for bystander exposure to antifouling products. It is considered that bystander exposure to antifouling products applied to the hull of large commercial vessels will be lower than for other workers, since spraying operations are generally avoided by others in the dry-dock, vessels do not emit zineb once antifouling product has settled on the ship surface and contact with wet surfaces by third parties is unlikely.

For amateur applications, passers by within a congested yard could contact the hulls of freshly treated boats. There is a very low risk of skin sensitization for bystanders if such exposure were repeated.

4.3 DATA REQUIREMENTS

The ACP agreed in 1999 that skin penetration data should be required and that PPE should be specified for professionals as detailed in full in Appendix 1. No data had yet been received when this complete review was considered in 2000. Following the ACP's discussions in 2000 the following further data requirements and conditions of approval were identified:

1. Provisional Approval should be allowed to continue for the professional use of antifouling products containing zineb at a maximum concentration of 20 % w/w, for application by brush, roller and spray, subject to the following:

    a. Professional operators should wear impermeable gloves of a type recommended by the antifouling manufacturer as suitable for use with the formulation. These gloves should be changed regularly, e.g. after one or two days' use. Operators should wear impermeable (and non-slip) footwear that protects the lower leg.

    b. The approval conditions appearing on professional-use products' Notices of Approval and Schedules should be reflected on product labels using the following precautionary phrases:

WEAR SUITABLE PROTECTIVE CLOTHING (COVERALLS OF A CONTRASTING COLOUR TO THE PRODUCT BEING APPLIED, BENEATH A DISPOSABLE COVERALL WITH HOOD), SUITABLE GLOVES, AND IMPERVIOUS FOOTWEAR THAT PROTECTS THE LOWER LEG.

DISPOSE OF PROTECTIVE GLOVES AFTER USE

If the product is to be applied by spray:

WEAR SUITABLE RESPIRATORY EQUIPMENT SUCH AS AIR-FED RESPIRATORY EQUIPMENT WITH COMBINED PROTECTIVE HELMET AND VISOR WHEN SPRAYING.

2. The potential for skin sensitisation and possibly for respiratory irritation as a result of the amateur use of antifouling products containing up to 20 % w/w zineb is sufficiently low that approval for application by brush and roller should be allowed to continue, subject to existing data requirements (Appendix 1) and users being required to wear gloves as a precautionary measure.

3. As the potential for dermal exposure during spraying by amateurs is much greater than during application by brush or roller, the ACP may wish to revoke approval for amateur application by spraying.

5 ENVIRONMENTAL FATE AND BEHAVIOUR

5.1 ZINEB

5.1.1 HYDROLYSIS

In 1985, a study was conducted to investigate the hydrolysis of zineb (purity 88 %), in the presence and absence of copper, in non-sterile distilled water and seawater at 23 ± 2 ^{o}C, pH unreported. The study did not follow any recognised guidelines, however, it was conducted to Good Laboratory Practice (GLP).

Nominal concentrations of 5 mg zineb ml^{-1} were prepared once in both distilled water and seawater (it was unclear whether the seawater was natural or artificial). These test solutions were mixed at 130 rpm for 24 h, and after this time any residues were removed. No analysis was performed to determine the composition of the residue. The remaining filtrate was approximately divided into two, and either treated with copper I oxide (Cu_2O) (purity 97 %) at a nominal concentration of ~0.67 mg ml^{-1} or left untreated, then returned to the mixer. At 24, 48 and 72 h an unreported number of samples of the test solutions were analysed for EBDC (ethylene bisdithiocarbamate) ions by gas chromatography with mass spectroscopy (GC/MS) following acid hydrolysis. EBDC ions appear to be one of the first products of zineb hydrolysis as the metal ion dissociates from the organo-metallic parent compound. It is assumed that the Author could analyse for EBDC ions more easily than for zineb, although no reason for analysis of a metabolite rather than the parent compound is given in the report.

The concentration of EBDC ions detected decreased with time (see Table 5.1), half-lives were not derived.

Table 5.1 Degradation of EBDC ions in distilled water and seawater

	µg EBDC ions ml^{-1}			
	in seawater		**in distilled water**	
Time after Cu_2O addition (h)	**with Cu_2O**	**without Cu_2O**	**with Cu_2O**	**without Cu_2O**
24	0.84	4.6	0.28	0.68
48	0.47	2.9	0.19	0.38
72	0.28	2.3	0.19	0.36

It was suggested that the presence of copper ions accelerated the rate of degradation, however, no mechanism for this was suggested. The Author also interpreted the tabulated results as showing poor recovery in distilled water, and suggested that this was due to differences in pH between this and seawater, although the pHs were not reported. Since the concentration of EBDC ions at 0 h and over the first 24 h have not been reported the true pattern of degradation cannot be seen. The information reported was insufficient to support the Author's conclusions (Unpublished, 1985a.).

A study was conducted by Klisenko and Vekshtein (1971) to investigate the hydrolysis of several EBDC pesticides, including zineb (purity unreported). The rate of hydrolysis and production of metabolites was studied at pH 3.8, 5.7, 7.0, 7.96 and 11.2, at 18 - 20 ^{o}C in the light. Concentrations of zineb and any metabolites were analysed using thin layer chromatography (TLC) with ultra-violet (UV) spectrophotometry. The rates of zineb hydrolysis followed first order kinetics and were dependent on hydrogen ion concentration (see Table 5.2). Unfortunately, since the tests were not conducted in the dark, it is possible that photolytic, as well as hydrolytic, degradation was being observed.

Table 5.2 Rate of hydrolysis of zineb

	pH				
	3.8	**5.7**	**7**	**7.96**	**11.2**
Hydrolysis rate constant (h^{-1})	0.5×10^{-1}	1.07×10^{-1}	7.2×10^{-3}	1.7×10^{-3}	7.1×10^{-4}
Half-life (h)	0.15	6.48	96.1	405.3	976

Zineb degraded to DIDT (5,6-dihydro-3H-imidazo (2,1-c) 1,2,4-dithiazole-3-thione), ethylene thiourea (ETU) and sulphur, relative amounts varying with pH. The half-life of zineb was 96.1 h at pH 7, 18 - 20 ^{o}C; and therefore this a.i. was considered to readily hydrolyse.

5.1.2 DEGRADATION IN SEAWATER

A study was conducted to investigate the degradation of zineb (purity unreported) in seawater, in the presence and absence of copper (Hunter and Evans, 1991a). Zineb in artificial seawater, with or without copper, was shaken (180 rpm) under constant illumination (photon flux density 130 µmol m^{-2} s^{-1}) at 18 ^{o}C for 96 h. Samples were taken at regular intervals and analysed using UV spectrophotometry, TLC and high performance liquid chromatography (HPLC). Zineb alone degraded very rapidly to DIDT, which was detected in the 0 h sample, and then, within 11 h ETU was detected. In the presence of copper the zineb again degraded immediately to DIDT, but then degraded to ethylene diisothiocyanate (EDI) and an unidentified compound.

5.1.3 LEACHING FROM A TREATED SURFACE

In 1985, a study was conducted to investigate the leaching of a formulation containing zineb (technical grade) from a treated glass surface. The study did not follow a specified guideline, however, it was conducted to GLP.

An antifouling product, with 3.3 % zineb incorporated, was applied to an area of 15 cm^2 on each of an appropriate number of abraded glass microscope slides. The slides were left at ambient temperature for the solvent to evaporate, and then left for a further 48 h at room temperature for the paint to harden.

Duplicate slides were each immersed in 100 ml distilled water (pH 7) or 100 ml UK seawater (pH 8.2). The solutions were maintained at 23 ± 2 ^{o}C on an orbital mixer (130 rpm) for 24 h. After this time all of the water was removed for analysis and replaced. This procedure was repeated at intervals over 53 d, although the length of time between sampling was increased as the study progressed. The samples were treated with stannous chloride in concentrated

hydrochloric acid liberating carbon disulphide (CS_2), which was then analysed by GC/MS. Calibration standards were used to calculate the amount of zineb present in each sample from the amount of CS_2 detected.

The reproducibility of the results was reported to be low, due to the low levels of CS_2 being detected. The amount of zineb leached into the distilled water (0.05 ± 0.03 µg cm^{-2} d^{-1}) and seawater (0.04 ± 0.02 µg cm^{-2} d^{-1}) did not vary significantly, and was relatively constant throughout the duration of the study. The Author dismissed a number of outlying results; for example, on day 32 one seawater replicate leached 0.49 µg zineb cm^{-2} d^{-1}. However, a tiny piece of paint was reported to be missing from the slide, probably explaining the high concentration detected. By day 53 it was reported that tiny blisters had appeared in the paint of all four slides, probably indicating that the paint was becoming detached from the glass.

No specific conclusions were made by the Author about the leaching of zineb from a glass surface. However, it was suggested that the low concentrations detected were due to the rapid rate of hydrolysis of zineb (Unpublished, 1985b).

A study was conducted by Hunter and Evans (1991b) to investigate the leaching of zineb from antifouling products. Seven paints containing varying levels of tributyltin oxide (TBTO), copper (I) oxide (Cu_2O) and zineb, and five paints containing just Cu_2O and zineb were applied to abraded formica panels. These panels were suspended, at various depths, from a raft in the Yealm Estuary, Newton Ferrers, Devon. The panels were sampled regularly over a 15-month period. Any silt build up was removed, and the panels were then left in seawater in the laboratory, for 22 h. After this time the seawater was analysed for zinc using atomic absorption spectroscopy (AAS), and this was assumed to be equivalent to the amount of zineb in the water. A product containing 8 % TBTO, 22 % Cu_2O and 21.6 % zineb leached >10 µg zineb cm^{-2} d^{-1} over the first seven months of the study. A second product containing 32 % Cu_2O and 32 % zineb leached >3.8 µg zineb cm^{-2} d^{-1} over the first eight months of the study. It was reported that most paints leached at a similar rate, however, specific leaching rates for the other 10 paints were not reported. It was noted that zineb hydrolysed rapidly, with the metabolites DIDT and EDI detected in the leachate. There was no mention of recording background levels of zinc in the estuarine water, potentially reducing the reliability of the analytical method.

5.1.4 NEWLY SUBMITTED ZINEB DATA

In May 1999 additional data were submitted in support of the use of zineb as a booster biocide in antifouling products. These data were assessed initially, and several reference papers reviewed. However, it was considered that they would not significantly change the environmental risk assessment, so full evaluations have not been included in this evaluation (Unpublished, 1997a, 1997b; Vonk, 1975; Bol *et al*., 1991; Ordelman *et al*., 1993).

5.2 MANCOZEB

5.2.1 STERILE HYDROLYSIS

In 1988, a study was conducted to investigate the hydrolysis of mancozeb (purity 92.5 %) at pH 5, 7 and 9, under sterile conditions and at 25.4 ± 1.1 ^{o}C for 96 h. The study followed US EPA FIFRA Guideline 161-1, and was conducted to GLP.

^{14}C-radiolabelled mancozeb at a concentration of ~6 mg ml^{-1} in acetone was initially prepared. 187 µl of this was mixed with 100 ml of each buffer (pH 5, 7 and 9), after allowing the acetone to evaporate, giving a nominal final test concentration of 11.2 µg ml^{-1}. The hydrolysis tests were carried out in triplicate, in the dark.

Test samples were analysed regularly for mancozeb and any metabolites using HPLC and TLC. The pH of the buffers remained stable throughout the study. The recoveries of ^{14}C were acceptable, with 104.8 ± 27.6 %, 101 ± 8.6 % and 119 ± 21.8 % at pH 5, 7 and 9 respectively. Samples were only required to be taken over the first 96 h, after which time the mancozeb had completely degraded hydrolytically to DIDT, ETU and ethylene urea (EU). The proposed pathway of hydrolytic degradation is shown in Figure 5.1.

The Authors suggested that hydrolysis via DIDT was catalysed by alkaline conditions, hence the detection of DIDT only at pH 7 (44.5 % applied radioactivity [AR] at 30 h) and 9 (93.8 % AR at 0 h). However, it is also possible that DIDT was not detected at pH 5 due to the speed of hydrolytic degradation.

Mancozeb was hydrolysed readily, with the rate of the reaction increasing as the acidity increased. Linear regression was used to derive half-lives of 2.2, 5.5 and 14.1 h at pH 5, 7 and 9 respectively (Unpublished, 1988a).

5.2.2 AEROBIC AQUATIC METABOLISM

In 1995, a study was conducted to investigate the degradation and metabolism of mancozeb (purity 88.5 %) in freshwater systems. The study was conducted to the German BBA Part IV: 5-1 (December 1990) guideline, and in consideration of US EPA FIFRA Guideline 171-4. The study was performed to GLP.

Water from a depth of 10 to 30 cm and sediment from the top 5 to 10 cm, were collected from a pond and river system in Switzerland (see Table 5.3 for characterisation). The water was filtered to 0.2 mm and the sediment sieved to 2 mm, then put into metabolism flasks. A system of ethylene glycol and sodium hydroxide traps were connected to the flasks. The flasks were equilibrated at 20 ± 1 ^{o}C for three weeks prior to application of the active ingredient (a.i.).

Figure 5.1 Proposed pathway of hydrolytic degradation of mancozeb

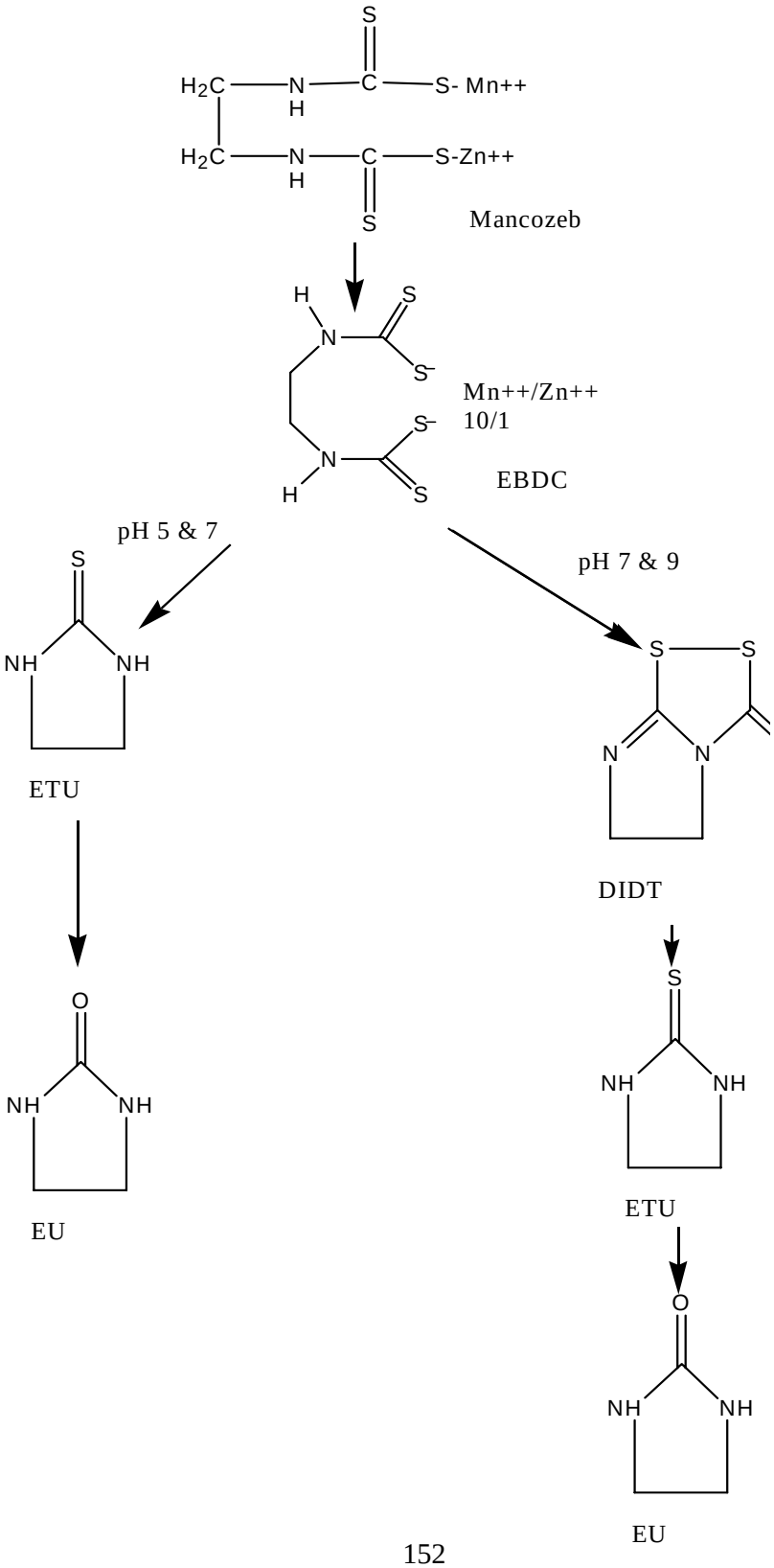


Table 5.3 Characteristics of the aquatic systems

	River		Pond	
Parameter	**Water**	**Sediment**	**Water**	**Sediment**
Temperature (^{o}C)	5.4	ND	7.15	ND
pH	8.13	6.85	7.38	6.61
Organic carbon (mg C l^{-1} water/g C 100 g^{-1} dry sediment)	2.6	1.35	1.1	5.03
Cation exchange capacity (meq 100 g^{-1} dry sediment)	ND	2.77	ND	7.05
Particle size distribution (USDA nomenclature)	ND	Sand:	ND	Loam:
Clay (% <2 µm)	ND	7	ND	5
Silt (% 2 - 50 µm)	ND	11.6	ND	35.8
Sand (% >50 µm)	ND	81.4	ND	59.2

ND- not determined

An appropriate number of flasks were treated with ^{14}C-radiolabelled mancozeb (35.1 mg l^{-1}), so that for each test system duplicate samples of water and sediment could be taken at each time point (0, 0.25, 1, 2, 7, 14, 30, 59 and 105 d). In addition, four untreated flasks were set up as controls. The aquatic systems were then ventilated with moistened air, and stirred continually.

The radioactive fractions in the sediment samples were extracted in acetonitrile and water or using soxhlet with methanol. The water and sediment fractions were characterised using liquid scintillation counting (LSC), HPLC and TLC. Recoveries during the test were good, 95.2 ± 2.8 % for the river and 93.8 ± 3 % for the pond.

The radioactivity level in the water decreased over the 105 d to 3.2 % AR in the river and 18.2 % AR in the pond. Radioactivity in the sediment peaked at 14 - 30 d (23.1 % AR in the river and 19.9 % AR in the pond), then decreased to 3.8 % AR in the river and 12.5 % AR in the pond. There were reported to be no differences between the river and pond systems.

Mancozeb was detected in the water compartment (peaking at 16.4 % AR in the river and 43.1 % AR in the pond at 6 h), but never in the sediment. Several metabolites were detected in the water and sediment in varying concentrations over the duration of the study (see Table 5.4), including five minor, unidentified compounds. The proposed metabolic pathway for mancozeb in aquatic systems is shown in Figure 5.2.

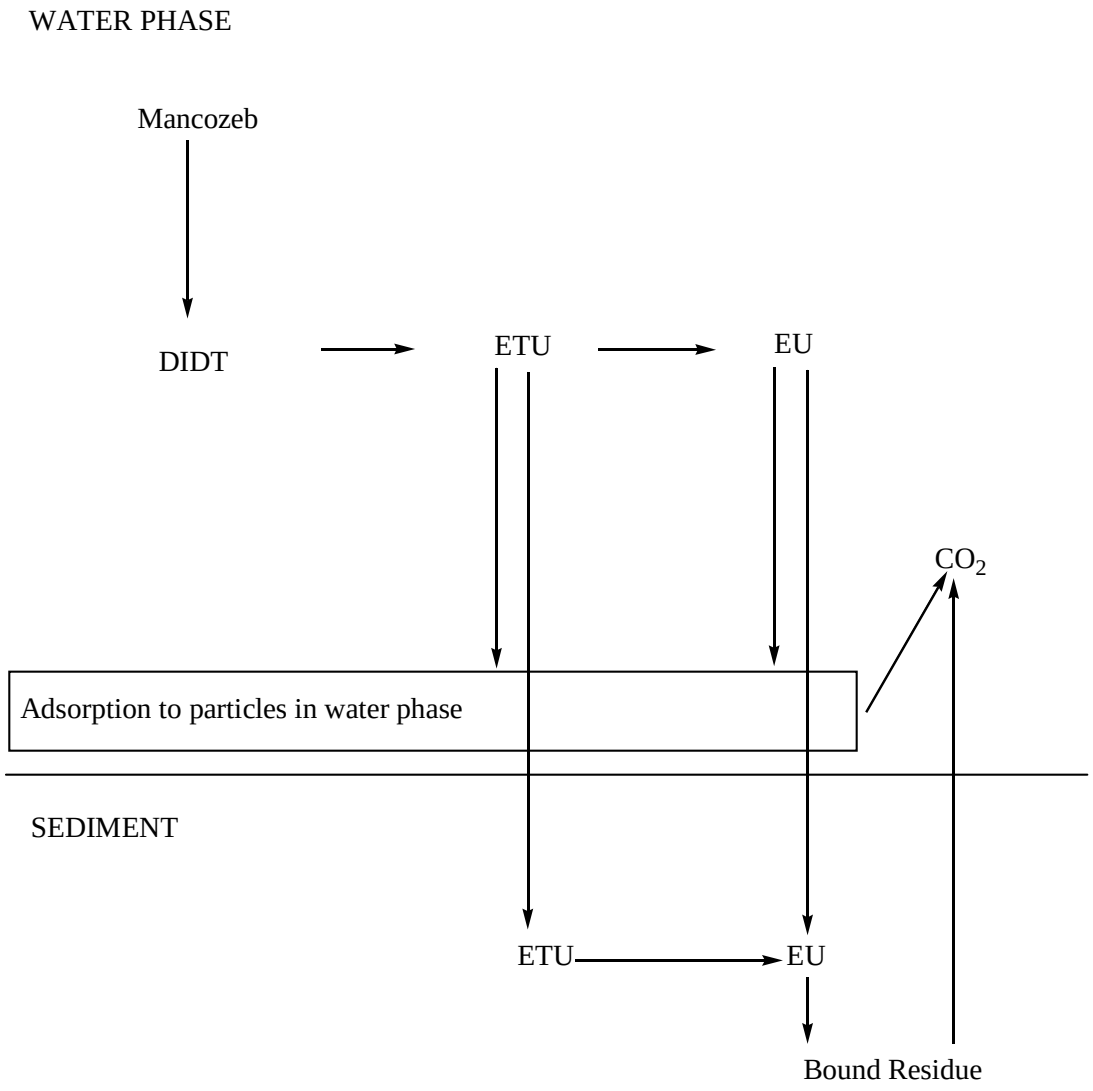


Figure 5.2 Proposed pathway of degradation of mancozeb in aquatic systems

Table 5.4 Metabolites detected in the water and sediment

Metabolite	Peak concentration (% AR)	River/Pond Water/Sediment	Day of peak
ETU	41.9	River water	0.25 (6 h)
	48.5	Pond water	1
	6.3	River sediment	2
	6.6	Pond sediment	14
DIDT	12.7	River water	0.25 (6 h)
	3.8	Pond water	0.25 (6 h)
	3.8	River sediment	2
	1.1	Pond sediment	2
EU	22.5	River water	30
	23.4	Pond water	59
	7.8	River sediment	30
	9.1	Pond sediment	30
Hydantoin	8.6	River water	14
	5.7	Pond water	14
	3	River sediment	14
	2.2	Pond sediment	14

After 105 d most of the mancozeb had undergone complete mineralisation (17.6 - 47.1 % AR), or was associated with the sediment as non-extractable residues (39.5 - 43.6 % AR). A validated computer program was used to derive the rate of disappearance of mancozeb and ETU (DT_{50} and DT_{90}) from the water and sediment (see Table 5.5).

Table 5.5 Degradation rates of mancozeb and ETU

		River		Pond	
		Water	**Sediment**	**Water**	**Sediment**
Mancozeb	**DT_{50} (d)**	0.2	-	0.1	-
	DT_{90} (d)	2	-	1.3	-
ETU	**DT_{50} (d)**	4	6.4	6.3	2
	DT_{90} (d)	13.3	21.1	21	22.3

The testing laboratory produced an amendment to the study report in 1996 at the request of the sponsor, to correct several mistakes. Consequently, the DT_{50} and DT_{90} values for mancozeb in the river and pond water were amended. The corrected values are presented in Table 5.5.

It was concluded that mancozeb was rapidly broken down in freshwater systems, with hydrolysis as the main route of degradation to the metabolites DIDT, ETU, EU etc. Further degradation was not considered by the Authors, but was assumed to be via biotic processes (Unpublished, 1995a and 1996).

5.2.3 DEGRADATION IN SOIL

5.2.3.1 AEROBIC METABOLISM

In 1972, a study was conducted to investigate the aerobic degradation of mancozeb (purity unknown) in soil.

The test soil, a silt loam (see Table 5.6), was prepared by drying, sieving to 0.19 cm and rehydrating to ~11 % moisture.

Table 5.6 Characteristics of the soil

Parameter	Silt loam
Mechanical analysis (%)	Sand - 38 Silt - 34 Clay - 28
Organic matter (%)	3.42
Cation exchange capacity (meq 100 g^{-1})	9.4
pH	6.4

^{14}C-radiolabelled mancozeb or ETU were mixed into the soil to give nominal concentrations of 10 and 20 µg a.i. g^{-1} soil, or 10 µg ETU g^{-1} soil. The latter two test concentrations were also applied to sterile soil to investigate the abiotic degradation of the two compounds. Three biometer flasks were set up for each test concentration, containing either 300 or 200 g of soil. The Author reported that controls were set up in a similar manner, but no further details were given. In addition, three flasks containing no soil were set up as blanks. Each biometer flask had a 0.5 N (0.5 M) sodium hydroxide trap for evolved carbon dioxide (CO_2).

The flasks were incubated at an unreported temperature for 33 or 170 d. The traps were sampled at regular intervals, and analysed for ^{14}C by liquid scintillation counting (LSC) and total CO_2 by titration with hydrochloric acid. Radioanalysis of the trapping solutions showed that >99 % of the ^{14}C was in the form of CO_2. The soil was analysed for residual ^{14}C (method unreported), but the results could not be assessed because they were reported without any units.

Similar levels of total CO_2 were produced by the micro-organisms in the control and those exposed to 10 µg g^{-1} soil mancozeb or ETU. However, less total CO_2 was produced by the micro-organisms exposed to 20 µg a.i. g^{-1} soil when compared to the control. The Author suggested that this was evidence of microbial inhibition at the higher concentration. However, an *in vitro* investigation into the toxicity of mancozeb to micro-organisms found in soil gave MICs (minimum inhibitory concentrations) ranging from $\leq$2 to >1000 µg a.i. l^{-1}. These results offered no support for the conclusion that mancozeb was toxic to soil micro-organisms at 20 µg a.i. g^{-1} soil, but not at 10 µg a.i. g^{-1}. Also, the total CO_2 results appear to have been miscalculated, therefore, little useful information could be drawn from them.

No mineralisation was seen under sterile conditions after 33 days. The Author concluded that any degradation observed was due to biotic processes. After 33 d 42.9 % of the applied a.i. (20 µg g^{-1} soil) and 52.6 % of the ETU were trapped as ^{14}C, and the half-lives of mancozeb

and ETU were estimated to be 50 and 22 d respectively. After 26 d 32 % of the applied a.i. (10 µg g^{-1} soil) was trapped as ^{14}C and after 170 d 57.1 %, therefore, at this concentration the half-life of mancozeb was estimated to be 90 d. In contradiction to the initial conclusion, the Author also suggested that the higher rate of degradation at the highest test concentration was due to increased activity of the micro-organisms in the soil. HSE has concluded that the concentration dependent degradation reported was most likely the result of differences in available carbon at the two a.i. concentrations. This study appears to show that the active ingredient and its metabolite are biodegradable, but via a slow process (Unpublished, 1972).

A study was conducted to investigate the aerobic degradation of mancozeb (purity 95 %) in soil (Doneche, B. *et al*, 1983). Four soils were treated with 10 mg mancozeb kg^{-1} and incubated at 21 ± 1 ^{o}C, in the dark, for 90 d. A repeat application was made to one soil 40 d after the initial treatment, and sterilised soil was also treated. Five days after treatment 40.3 - 64.9 % of the applied a.i. had degraded in the non-sterilised soil, and after 90 d $\geq$96.8 % had degraded. After 5 d in the sterilised soil 33.9 - 40.2 % of the applied a.i. had degraded, and after 90 d 54 - 78 % had degraded. This suggested that abiotic processes initially drove degradation, but later stages involved biotic processes. It was also reported that the degradation rate increased after repeat applications, especially during the first five days following treatment. This suggested that the initial stage of degradation was concentration dependent, therefore, supporting the previous conclusion that this was an abiotic process.

5.3 METABOLITES

5.3.1 MOBILITY IN SOIL

In 1988, a study was conducted to investigate the leaching characteristics of aerobically aged mancozeb (purity 87.4 %) in soil. The study was conducted to US EPA FIFRA Guideline 163-1, and to GLP.

Four US soils with differing characteristics (see Table 5.7) were prepared by drying and sieving to 2 mm.

Table 5.7 Characteristics of the soil

Parameter	Sandy loam	Silt loam (I)	Clay loam	Silt loam (II)
% Organic matter	0.4	2	3	4.5
Field capacity (1/3 bar moisture %)	9.29	28.61	23.65	28.9
Cation exchange capacity (meq 100 g^{-1})	7.5	6.1	16.9	7.9
% Sand	76	12	26	28
% Silt	18	62	46	54
% Clay	6	26	28	18
pH	7.8	6.1	6.9	6.2

A stock solution of ^{14}C-radiolabelled mancozeb in methanol (57.03 µg a.i. ml^{-1}) was prepared and 14 ml mixed into 20 g of each soil type. The methanol was evaporated off under a stream of nitrogen, and the spiked soil blended with an additional 60 g of soil, giving a final concentration of 13.3 µg ml^{-1}. This soil was then aerobically aged for 24 h (the half-life of

mancozeb, as stated in this study) at 25 ± 1 ^{o}C, in the dark, in a metabolism vessel. After aging, the mancozeb content of the soil was found to be <5 % of that applied, using TLC.

Four replicate aluminium columns (91.44 by 7.62 cm) were loaded to a depth of 30.48 cm for each soil type. Three soil columns had spiked, aerobically aged soil placed on the top, and the fourth was used as a control (no further details reported). Each column was treated with 2320 ml of deionised water.

Once all of the standing water had drained through the column the leachate was sampled. The amount of radioactivity in the leachate and any volatiles, trapped from the soil or leachate, was measured using LSC. The soil columns were cut into twelve equal segments that were also analysed for ^{14}C content.

The amount of time for the water to leach through the soil varied from 1 d (sandy loam and silt loam [II]) to 34 d (clay loam) (no additional results were given for the clay loam). See Table 5.8 for further results. In a deviation from the EPA guideline, K_d values for the soils were not calculated.

Table 5.8 Distribution of ^{14}C in soil and leachate

	Sandy loam	**Silt loam (I)**	**Silt loam (II)**
% of AR in leachate (mean and sd)	19.1 ± 0.85	8.7 ± 0.45	4.2 ± 0.21
% of AR in soil (mean and sd)	77.8 ± 3.55	98.9 ± 12.94	90.2 ± 19.96
% of AR in top 2.5 cm of soil (mean and sd)	56.8 ± 5.25	84.2 ± 10.05	83 ± 19.08
% of AR trapped as volatile compounds (mean and sd)	0	0	0

A large proportion of the applied radioactivity remained in the soil, especially in the top 2.5 cm. Only a small proportion was detected in the leachate, and none was released as volatile compounds. From these results it appeared that the metabolite(s) of mancozeb were relatively immobile in soil (Unpublished, 1988b).

5.3.2 FATE AND BEHAVIOUR IN SOIL

In 1995, a study was conducted to investigate the behaviour of ethylene thiourea (ETU) (purity unreported) in soil. The aim of the study was to develop a soil extraction method, to investigate the adsorption of ETU onto soil and its aerobic degradation in soil. The behaviour of ETU was initially studied in the laboratory, and then in the field. The study did not follow any guidelines, however, it was conducted to GLP.

5.3.2.1 EXTRACTION OF ETU FROM SOIL

A solution of thiourea (TU) was added to samples of soil (type used not reported) treated with ^{14}C-radiolabelled ETU (the TU was used to stabilise the ETU, as well as being the extraction solvent). This mixture was shaken, centrifuged and the supernatant analysed directly by LSC, or, after being cleaned up by solid phase extraction (SPE), was analysed by HPLC.

Immediate extraction yielded average recoveries of 85 %, the Authors considered this acceptable. However, after storage (-20 ^{o}C/4 ^{o}C) or air-drying, extraction recoveries were in the range of only 10 - 25 %.

5.3.2.2 ADSORPTION (COLUMN LEACHING METHOD)

Soil collected from two different regions of the Netherlands was used for this test. The first soil type was dune sand on top of clay from a northern location. The second came from a more southerly region of the Netherlands. This was a more humic sandy soil that had been treated with EBDC pesticides 9 weeks prior to the study. See Table 5.9 for characteristics of both types of soil.

Table 5.9 Characteristics of the soil

	Dune sand on clay soil			**Humic, sandy soil**	
Physical/chemical parameters	0 - 20 cm	20 - 40 cm	40 - 60 cm	0 - 30 cm	30 - 60 cm
% moisture pF2 (field capacity)	12.6	12.9	15	15.2	N/A
Density (g l^{-1})	1147.5	1128.5	1199	1238	N/A
Clay content, lutum <2 µm (%)	2.5	N/A	N/A	2.6	2.4
pH KCl	7.1	N/A	N/A	5	4.9
Organic matter content (%)	1.6	N/A	N/A	3.3	0.6

N/A - not analysed

Duplicate samples from the different layers of each soil were put into stainless steel columns (2.2 cm wide by 15 cm long). The soil was equilibrated with 0.01 M calcium chloride ($CaCl_2$), followed by a solution of ^{3}H (a non-sorbing tracer), ^{14}C-radiolabelled ETU and non-radiolabelled ETU dissolved in $CaCl_2$. The calcium chloride solution was pumped through the soil at a rate of $\leq$10 cm of precipitation a day. The length of the test period was not reported. The leachate was collected in 1 ml fractions, and analysed using LSC. The adsorption of ETU onto the soil was calculated from the difference in the amount of ^{3}H and ^{14}C in the leachate. This method was apparently chosen to limit the effects of ETU degradation on the test. However, it was not made clear why this method should alleviate such a problem.

The calculated adsorption coefficients (see Table 5.10) indicated that adsorption of ETU was low.

Table 5.10 Adsorption coefficients

	Dune sand on clay soil			Humic, sandy soil
	0 - 20 cm	20 - 40 cm	40 - 60 cm	0 - 30 cm
K_d (l kg^{-1})	0.05	0.04	0.01	0.05
K_{om} (l kg^{-1})	3	2.6	1	1.5

K_d - the soil/water distribution ratio
K_{om} - the adsorption coefficient with respect to the organic matter content

Therefore, ETU appeared to be mobile in sandy soil.

5.3.2.3 AEROBIC DEGRADATION

^{14}C-radiolabelled ETU (1.06 mg kg^{-1}) was applied to 25 g samples from the soil layers detailed in Table 5.9. The number of replicates used was not reported. The soil was incubated at 20 ^{o}C, and samples taken regularly over 168 h. The ETU was extracted and the amount of ^{14}C-ETU determined using LSC (recoveries 70 - 106 %) and HPLC (recoveries unreported). The percentage of ETU recovered was plotted against time, and $DT_{50/90}$ values derived using first order kinetics (see Tables 5.11 and 5.12).

Table 5.11 Degradation of ETU in the dune sand on clay soil

	LSC analysis		HPLC analysis	
	DT_{50} (h)	**DT_{90} (h)**	**DT_{50} (h)**	**DT_{90} (h)**
0 - 20 cm	7	24.5	3.8	12.7
20 - 40 cm	8	27	4.4	14.8
40 - 60 cm	11	36.5	5	17.2

Table 5.12 Degradation of ETU in the humic, sandy soil

	LSC analysis	HPLC analysis
	DT_{50} (h)	**DT_{50} (h)**
0 - 30 cm	24	1.3

HPLC analysis led to the derivation of lower half-lives, either because not all of the ^{14}C in the extract was in the form of ^{14}C-ETU, or some of the ^{14}C-ETU was bound to soil particles which were removed during the SPE. It was concluded that the differences in half-lives (derived by HPLC) between the two soils may have been due to slight variation in methodology, or because the humic, sandy soil had previously been treated with EBDC pesticides allowing for physiological adaptation of the soil micro-organisms to ETU. However, this variation may also have been due to physicochemical differences, e.g. pH, between the two soils.

5.3.2.4 THE BEHAVIOUR OF ETU IN THE FIELD

The field study was conducted at the dune sand on clay site (see Table 5.9 for the soil characteristics). Tulips were being grown on the field, which was treated with a total of 26.4 kg mancozeb ha^{-1} over a 12-week period.

Random 20 cm soil core samples were taken after each application, however, the time between application and sampling was not reported. Water samples pumped up from ~75 cm below the groundwater level were taken at the beginning and end of the study. All samples were stored frozen or refrigerated. Towards the end of the sampling period the results of the extraction experiment were obtained, and it was realised that analysis of the stored samples would give very poor recoveries. Therefore, the samples taken after the final application were extracted in the field, and were the only samples analysed. Also, after this application, four untreated soil samples were spiked and extracted in the field. All extracts were cleaned up in the laboratory using SPE cartridges, and analysed using HPLC.

Direct extraction in the field gave quite low recoveries, but the reproducibility was good (64.22 ± 2.22 %). Varying concentrations of ETU were found in the different soil layers (see Table 5.13).

Table 5.13 Concentration of ETU extracted from field soil after application of mancozeb

	Amount of ETU in soil, (µg kg^{-1}) (mean and standard deviation)
0 - 10 cm	42.78 ± 10.22
10 - 20 cm	4.54 ± 6.32
20 - 30 cm	22.54 ± 18.18
30 - 40 cm	7.14 ± 20.20

The amount of ETU in the groundwater increased after the application of mancozeb from 0.10 µg l^{-1} to 0.88 µg l^{-1}. The Authors concluded that this was evidence of leaching of ETU through the soil. However, this is not a large rise considering the amount of mancozeb applied to the soil. The low levels of ETU in groundwater, and the lack of results over time do not lend support to the Authors' conclusions.

The laboratory experiments indicated that the ETU was highly mobile and underwent rapid biodegradation in soil, however, this did not appear to happen in the field. The Authors concluded that after being transported into the lower levels of the soil conditions for degradation became less favourable, however, there were no data to support this (Unpublished, 1995b).

5.4 SUMMARY OF ENVIRONMENTAL FATE AND BEHAVIOUR DATA

5.4.1 ZINEB

Two studies that were conducted to GLP did not follow recognised guidelines. The fate and behaviour of zineb can be summarised as follows.

A hydrolysis study did not generate a half-life, or any other useful information that could be used to assess the stability of zineb in water. However, in a published paper (Klisenko and

Vekshtein, 1971) it was shown that zineb was hydrolytically unstable with a half-life of 96.1 h at pH 7, 18 - 20 ^{o}C. The metabolites DIDT, ETU and sulphur were produced as a result of hydrolytic degradation at a range of pHs (3.8 - 11.2). A second published study (Hunter and Evans, 1991a) confirmed that zineb hydrolysed readily in seawater. Also, the route of degradation varied in the presence and absence of copper ions, with ETU the major metabolite without copper, and EDI with copper.

A study to investigate the leaching of zineb from glass plates, yielded mean rates of 0.05 ± 0.03 µg a.i. cm^{-2} d^{-1} in distilled water and 0.04 ± 0.02 µg a.i. cm^{-2} d^{-1} in seawater. The low leaching rates were attributed to the instability of zineb in water. From a second, published leaching study (Hunter and Evans, 1991b) higher rates of >3.8 and >10 µg a.i. cm^{-2} d^{-1}, from paints containing Cu_2O and zineb, and TBTO, Cu_2O and zineb respectively, were derived. The metabolites DIDT and EDI were also detected in the leachate.

5.4.2 MANCOZEB

Most of the studies submitted for mancozeb were performed to GLP, and half also followed recognised guidelines, mainly those of the US EPA. The fate and behaviour of mancozeb (as a model for zineb) can be summarised as follows.

Mancozeb was shown to be hydrolytically unstable, with half-lives of 2.2, 5.5 and 14.1 h at 25 ^{o}C, and pH 5, 7 and 9 respectively. Several metabolites, DIDT, ETU and EU, were identified. A second study showed that mancozeb degraded rapidly in the water compartment of a freshwater-sediment system, with a DT_{50} of 0.1 - 0.6 d in water at pH 7.4 - 8.1, 5.4 - 7.2 ^{o}C. The a.i. was never detected in the sediment compartment. Several metabolites, including DIDT, ETU and EU, were found in both the water and sediment. ETU was the predominant metabolite, and had a DT_{50} of 4 - 6.3 d in water, and 2 - 6.4 d in sediment, at pH 6.6 - 6.9. It was concluded, from this study, that the initial route of degradation in an aquatic system was hydrolysis.

Mancozeb appeared to biodegrade slowly in soil, with half-lives of ~90 and ~50 d at 10 and 20 µg a.i. g^{-1} soil respectively. The metabolite ETU biodegraded a little more rapidly with a half-life of ~22 d at 10 µg g^{-1} soil. Mineralisation did not occur under abiotic conditions within 33 d. In a published study (Doneche, *et al*., 1983) it was suggested that abiotic processes initially drove aerobic degradation in soil, but after about 5 d biotic processes became more important.

5.4.3 METABOLITES

Two studies were submitted which investigated the mobility of metabolites of mancozeb. Both were performed to GLP and one also followed US EPA guidelines.

The mobility of aerobically aged mancozeb in soil was low with 57 - 83 % AR being detected in the top 2.5 cm of the soil column, and only 4 - 19 % found in the leachate. The ^{14}C-radiolabelled residues detected were not characterised, however, the aged soil contained <5 % a.i. when applied to the column, therefore, it is unlikely that much of the detected residue was mancozeb. A second study investigated the aerobic degradation and mobility of the metabolite ETU in soil. The derived half-life was 1.3 - 5 h, considerably lower than that calculated in the previous study. A K_{om} of 1 - 3 l kg^{-1} was calculated, indicating that the

metabolite was very mobile in soil. It was shown, in the field, that ETU reached a maximum depth of 40 cm in the soil profile, with most of the applied ETU being detected at 10 cm. This result did not support the findings of the previous mobility study that indicated that metabolite(s) of mancozeb were not particularly mobile in soil. However, these studies are probably not comparable since the ^{14}C was not characterised in the first study, leaving it unclear as to which metabolite(s) were being investigated; also two different methodologies were used.

5.5 ASSESSMENT OF THE READ-ACROSS ARGUMENT

5.5.1 INTRODUCTION

Environmental fate and behaviour data in support of the use of zineb as an antifouling booster biocide was submitted. A reasoned argument which stated that the structure and properties of mancozeb were similar enough to justify read-across between the two chemicals when assessing environmental hazard data was also submitted.

5.5.2 CONSIDERATION OF THE READ-ACROSS ARGUMENT

It was accepted that zineb and mancozeb have extremely similar structures, both being co-polymeric compounds comprising of chains of $EBDC^{2-}$ ions and metal ions (zinc in zineb, and zinc and manganese in a ratio of 10:1 in mancozeb).

Zineb and mancozeb appeared to have similar physicochemical properties, e.g. water solubility, vapour pressure and partition coefficient, which suggested that they might then behave in a similar manner in the environment. Zineb and mancozeb both degraded rapidly in water, although zineb appeared to be slightly more stable than mancozeb, however, both initially dissociated to metal and $EBDC^{2-}$ ions. Evaluation of studies on the degradation of zineb (Hunter and Evans, 1991a) and mancozeb (Unpublished, 1995a) in water to primary metabolites highlighted the similar routes of degradation that these compounds took.

It was noted that although the referenced papers supported most statements in the read-across document, not all were. For example, it was stated that ‘zinc salts of dialkyldithiocarbamates are more stable than corresponding manganese salts’. On evaluation the reference (Wickbold, 1956) did not support this statement, and it was also unclear as to its relevance to zineb and mancozeb. Also, for example, there was reference to Braibanti *et al*., (1969). The read-across argument referred to this paper in support of the statement that ‘long-range bonding interactions between Zn and EBDC subunits and p-interactions between Zn *d*-orbitals (both to its sulphur neighbours and to adjacent polymer units) and EBDC subunits are at least partially responsible for this stabilising effect that Zn has on the dissolution behaviour of the polymer material’. However, this paper primarily discussed the structure of hydrazinium hydrazinedithiocarboxylate, and although it did briefly compare this compound to dithiocarbamates no support for the preceding statement could be found. Mineralisation was also discussed in some detail in the read-across document, however, HSE did not consider that a sound argument on the similarities in this process for the two compounds had been presented.

In conclusion, the reasoned argument that zineb and mancozeb were similar enough in structure and properties to support their approval of the use of zineb in antifouling products (AFPs) with mancozeb data was accepted.

6 ECOTOXICOLOGY

6.1 ZINEB

6.1.1 TOXICITY TO SOIL PROCESSES

A study was conducted by Torstensson *et al.*, (1992) to investigate the effect of zineb on nitrification in soil. Soil slurries were treated with several (unreported) concentrations of zineb, and shaken at 175 rpm, 25 ^{o}C for an unspecified length of time. Samples were taken at regular intervals, and the rate of ammonium oxidation was derived from a measurement of the accumulated nitrite. An EC_{50} and NOEC were calculated (see Table 6.1).

Table 6.1 Toxicity of zineb to ammonium oxidation

	Soil I	**Soil II**
EC_{50} (µg a.i. g^{-1} dry weight of soil)	3.6	16
NOEC (µg a.i. g^{-1} dry weight of soil)	0.4	2.5

Zineb appeared to inhibit this step of nitrification, being toxic to the soil micro-organisms involved.

6.1.2 GROWTH INHIBITION OF FRESHWATER ALGAE

A study was conducted by van Leeuwen, *et al*., (1985a) to investigate the acute toxicity of zineb (purity 95 %) to the freshwater algae *Chlorella pyrenoidosa*. Triplicate algal suspensions with a starting density of 1 x 10^{8} cells l^{-1} were exposed to unspecified test concentrations of zineb, and incubated at 20 ± 1^{o}C, light intensity 7.5 W m^{-2} with constant mechanical shaking. A Coulter Counter was used to monitor the number of cells in suspension. After 96 h exposure the specific growth rate of the cells was calculated using the method described in OECD guideline 201; effects on inoculum, reproduction and yield were also measured, however, the methods used were not reported. A 96 h EC_{50} (inoculum) of 1.8 mg l^{-1} was determined.

6.1.3 ACUTE TOXICITY

6.1.3.1 TOXICITY TO FRESHWATER INVERTEBRATES

In 1985, a 48 h acute toxicity test was performed using zineb (purity unreported) against *Daphnia magna*, under static conditions. The test did not follow a specified guideline, however, it was conducted to GLP.

Following a range-finding study, four replicates of five daphnids (6 - 24 h old) were exposed to each nominal concentration of 1, 1.7, 3.1, 5.6 or 10 mg zineb l^{-1}, prepared in dechlorinated water. Four replicates of five daphnids were also exposed to dilution water only as a control. The test conditions were maintained at 21 ± 1 ^{o}C, pH 7.6 - 7.8, dissolved oxygen concentration 7.2 - 8.3 mg l^{-1} and a water hardness of 66 mg $CaCO_3$ l^{-1}. The daphnids were kept in a 16 h light photoperiod, and were not fed for the duration of the test. Observations for mortalities were made at 24 and 48 h.

A NOEC was not derived for this study. Probit analysis was used to calculate an EC_{50} (see Table 6.2).

Table 6.2 Acute toxicity of zineb to *D. magna*

Time (h)	Nominal EC_{50} (mg a.i. l^{-1}) (95 % confidence limits)
24	5.7 (3.6 - 9.1)
48	1.4 (1 - 2)

Therefore, the 48 h EC_{50} for *D. magna* was calculated to be nominal 1.4 mg zineb l^{-1} (Unpublished, 1985a).

A study was conducted by van Leeuwen, *et al.,* (1985a) to investigate the acute toxicity of zineb (purity 95 %) to *D. magna*. The daphnids were exposed to unspecified test solutions of zineb as per OECD guideline 202. In a modification to the guideline fresh stock solutions were prepared daily, and the test solutions renewed daily. Also the daphnids were fed *C. pyrenoidosa* during the test. A 48 h EC_{50} of 0.97 mg l^{-1} (95 % confidence limits of 0.56 - 1.8 mg l^{-1}) was derived using the Litchfield and Wilcoxon method.

6.1.3.2 TOXICITY TO MARINE INVERTEBRATES

In 1985, a 96 h acute toxicity test using zineb (purity unreported) was performed against brown shrimp (*Crangon crangon*), under semi-static conditions. The study did not follow a specified guideline, however, it was conducted to GLP.

Following a range-finding test, nominal concentrations of 39, 59, 89, 133 and 200 mg a.i. l^{-1} were prepared in synthetic seawater from a stock of zineb in deionised water and a surfactant. Groups of 18 animals were exposed to each of the test solutions and a dispersant control. The shrimp were kept at 15 ± 1 ^{o}C with a 16 h light photoperiod. The test solutions were maintained at pH 7.8 - 8.8, dissolved oxygen content 7.2 - 10 mg l^{-1} and a salinity of 30 - 30.8 %. Observations were made daily for mortality and signs of abnormal behaviour. After 96 h exposure the weights and lengths of the shrimp were measured.

The lengths and weights of individual shrimp were presented in the report, however, no statistical analysis was used on the data and the Authors did not discuss the results. It was reported that at higher doses a white discoloration to the exoskeleton was observed, however, the specific concentrations involved were not reported so this information could not be used to derive a NOEC.

Four deaths occurred at nominal 133 mg zineb l^{-1}, and two at nominal 59 mg zineb l^{-1}. There were no deaths in the control group or any other test solution, therefore, a LC_{50} could not be calculated (Unpublished, 1985b).

6.1.3.3 TOXICITY TO FRESHWATER FISH

In 1979, a 96 h static acute toxicity test was carried out, using technical zineb, against rainbow trout (*Oncorhynchus mykiss* previously *Salmo gairdneri*).

Groups of ten fry (6 ± 1 cm long) were exposed to nominal concentrations of 0, 10, 20, 25.1, 30, 35 or 40 mg zineb l^{-1}. No details were provided on the nature of the control used. The oxygen content and pH of the test solutions were not reported. The test vessels were aerated, and maintained at 15 ± 1 ^{o}C, with an 8 h light photoperiod. The fish were not fed throughout the test period. They were observed daily for mortality and sub-lethal effects.

It was reported that dizziness was observed in some fish after a few hours' exposure, however, no more details were provided and a NOEC was not derived. Nominal LC_{50} values were derived using the Litchfield and Wilcoxon method (see Table 6.3).

Table 6.3 Acute toxicity of zineb to rainbow trout

Time (h)	Nominal LC_{50} (mg a.i. l^{-1}) (95 % confidence limits)
48	35 (31.2 - 39.2)
72	33 (30.3 - 36)
96	29 (28 - 30)

Therefore, the 96 h LC_{50} for rainbow trout was calculated to be nominal 29 mg zineb l^{-1} (Unpublished, 1979).

In 1985, two further 96 h static acute toxicity tests using zineb (purity unreported) were performed against rainbow trout and bluegill sunfish (*Lepomis macrochirus*). The studies did not follow specified guidelines, however, they were conducted to GLP.

Following range-finding tests solutions were prepared in dechlorinated tap water from a stock of zineb in deionised water and Tween 80 (see Table 6.4).

Table 6.4 Nominal exposure concentrations of zineb

Nominal concentrations (mg a.i. l^{-1})	
Rainbow trout	**Bluegill sunfish**
39	10
59	17
89	31
133	55
200	100

Groups of 10 animals of each species were exposed to each of the test solutions and a dispersant control. The test conditions were maintained as shown in Table 6.5.

Table 6.5 Test conditions

	Rainbow trout	Bluegill sunfish
Temperature (^oC)	13 ± 1	23 ± 1
pH	7.3 - 8	7.3 - 7.9
Dissolved oxygen concentration (mg l^{-1})	7 - 10.2	8.1 - 8.6
Water hardness (mg $CaCO_3$ l^{-1})	80	86

All fish were kept in a 16 h light photoperiod. Observations were made at 3, 6 and 24 h, and then daily, for mortality and signs of abnormal behaviour. After 96 h exposure the weights and lengths of the fish were measured.

The lengths and weights of individuals from both fish species were presented in the report, however, no statistical analysis was used on the data and the Authors did not discuss the results. Abnormal swimming was observed in rainbow trout at the higher concentrations, however, the specific concentrations were not reported so this information could not be used to establish a NOEC for this species. No sub-lethal effects were reported for bluegill sunfish.

Probit analysis was used to derive all LC_{50} values (see Table 6.6), which were based on nominal test concentrations. It was noted in the report that 95 % mortality at 100 mg a.i. l^{-1} was assumed to derive the endpoints for the bluegill sunfish. It was not clear why this was necessary since there was 100 % mortality at this concentration by 24 h, and the dose-response curve was not steep.

Table 6.6 Acute toxicity of zineb to fish

	Nominal LC_{50} (mg a.i. l^{-1}) (95 % confidence limits)	
Time (h)	**Rainbow trout**	**Bluegill sunfish**
48	170 (140 - 207)	37 (29 - 47)
72	99 (81 - 120)	37 (29 - 47)
96	42 (27 - 66)	35 (27 - 45)

Therefore, the 96 h LC_{50} values were calculated to be nominal 42 and 35 mg zineb l^{-1} for the rainbow trout and bluegill sunfish respectively (Unpublished, 1985c).

A study was conducted by van Leeuwen, *et al*., (1985a) to investigate the acute toxicity of zineb (purity 95 %) to the guppy (*Poecilia reticulata*). The fish were exposed to unspecified test solutions of zineb as per OECD guideline 203. In a modification to the guideline fresh stock solutions were prepared daily, and the test solutions renewed daily. A 96 h LC_{50} of 7.2 mg l^{-1} (95 % confidence limits of 5 - 10.3 mg l^{-1}) was derived using the Litchfield and Wilcoxon method.

6.1.3.4 TOXICITY TO MARINE FISH

In 1985, a 96 h static acute toxicity test using zineb (purity unreported) was performed against juvenile plaice (*Pleuronectes platessa*). The study did not follow a specified guideline, however, it was conducted to GLP.

Nominal concentrations of 17, 26, 39, 59, 89, 133 and 200 mg a.i. l^{-1} were prepared in synthetic seawater from a stock of zineb in deionised water and a surfactant. Groups of 10 juvenile plaice were exposed to each of the test solutions and a dispersant control. The fish were kept at 14 ± 1 ^{o}C with a 16 h light photoperiod. The test solutions were maintained at pH 7.4 - 8.9, salinity 30.1 - 31.8 %, with a dissolved oxygen concentration of 6.4 - 10.2 mg l^{-1}. Observations were made at 3, 6, 12 and 24 h, and then daily, for mortality and signs of abnormal behaviour. After 96 h exposure the weights and lengths of the fish were measured.

The lengths and weights of individual juvenile plaice were tabulated in the study, however, no statistical analysis was used on the data and the Authors did not discuss the results. Abnormal swimming was reported as being observed in juvenile plaice at the higher concentrations, however, the specific concentrations involved were not reported so this information could not be used to derive a NOEC.

It was not clear how the LC_{50} values (see Table 6.7) were derived, however, it was reported that precise values could not be calculated due to the steep dose-response curve.

Table 6.7 Acute toxicity of zineb to juvenile plaice

Time (h)	Nominal LC_{50} range (mg a.i. l^{-1})
48	40 - 60
72	26 - 40
96	26 - 40

Therefore, the 96 h LC_{50} for juvenile plaice was calculated to be in the range of nominal 26 - 40 mg zineb l^{-1} (Unpublished, 1985d).

6.1.3.5 ACUTE ORAL TOXICITY TO BIRDS

In 1976, 10 d acute oral toxicity tests were carried out using technical zineb against quail, pheasants (Formosana strain) and wild ducks.

An unreported number of unspecified doses of zineb in distilled water were prepared. Groups of 10 quail, 6 ducks and 6 pheasants were each administered a single oral dose directly into the stomach, after fasting for 6 h. It was not reported whether controls were used. During the test the birds were kept outside in 'appropriate enclosures'; more specific test conditions were not reported. All animals received feed and water *ad libitum* throughout the observation period of 10 d.

No signs of sub-lethal toxicity were observed, and there were no mortalities in any species. The Authors, therefore, reported that the LD_{50} for the quail, pheasant and wild duck was

>10 mg zineb kg^{-1}. The study was reported in insufficient detail to have confidence in the quality of the LD_{50} (Unpublished, 1976).

In 1985, a 14 d acute oral toxicity test was carried out using zineb (purity unreported) against 16-week-old bobwhite quail (*Colinus virginianus*). The study did not follow a specified guideline, but was conducted to GLP.

Following a range-finding test nominal doses of 500, 750, 1000, 1500 and 2000 mg zineb kg^{-1} body weight were prepared in gelatine capsules. Groups of 10 birds (5 of each sex) each received one capsule, directly into the crop, after overnight fasting. Ten control birds were given one empty capsule each. During the test the birds received feed and water *ad libitum*. The environmental conditions were maintained at 16 - 28 ^{o}C, 32 - 62 % r.h., with an 18 h light photoperiod. The birds were monitored twice daily for signs of abnormal behaviour and mortality. Body weight was recorded every 3 d, and food consumption was recorded daily. At the end of the test all surviving birds were sacrificed and a full macroscopic examination carried out.

There were no significant treatment-related effects on body weight, food consumption or gross pathology, therefore, the NOEL was nominal >2000 mg zineb kg^{-1} body weight.

One animal, from the control group, was killed *in extremis*, on day 4, after exhibiting an encrustation on the head and feather loss on the neck. A necropsy of this animal revealed no other clinical abnormalities. There were no mortalities in any other group during the 14 d test, therefore, a LD_{50} could not be established for the bobwhite quail (Unpublished, 1985e).

6.1.3.6 ACUTE DIETARY TOXICITY TO BIRDS

In 1985, two 8 d acute dietary toxicity tests were carried out using zineb (purity unreported) against 14-day-old bobwhite quail and 7-day-old mallard duck (*Anas platyrhynchus*). The study did not follow a specified guideline, but was conducted to GLP.

Following a range-finding test, against the bobwhite quail, nominal doses of 1000, 1800, 3000, and 5000 ppm zineb were prepared in diet for both species. Groups of fifteen birds received treated or untreated feed, plus water, *ad libitum* for 5 d. All dose groups were then fed untreated diet for a further 3 d. The environmental conditions were maintained at 16 - 29 ^{o}C, 38 - 56 % r.h. for the bobwhite quail and 20 - 33 ^{o}C, 58 - 66 % r.h. for the mallard duck, with an 18 h light photoperiod. The birds were monitored twice daily for signs of sub-lethal effects and mortality. Body weight was recorded on days 0, 5 and 8, and food consumption was recorded daily. At the end of the test all surviving birds were sacrificed and a full macroscopic examination carried out.

There were no clinical signs of toxicity, no significant effects on body weight or food consumption, and no significant pathological changes were observed at necropsy. Therefore, the NOEC for the bobwhite quail and mallard duck was nominal >5000 ppm zineb. There were no treatment related mortalities for either species, therefore, a LC_{50} could not be established (Unpublished, 1985f).

6.1.4 CHRONIC TOXICITY

6.1.4.1 TOXICITY TO FRESHWATER INVERTEBRATES

A study was conducted by van Leeuwen, *et al*., (1985b) to investigate the effect of zineb (purity 95 %) on survival, reproduction and growth in *D. magna*.

Five groups of 10 neonate daphnids (<24 h old) were each exposed to unspecified nominal test solutions of zineb and a test medium control (filtered and sterilised lake water) for 21 d. The daphnids were kept at 20 ± 0.5 ^{o}C in a constant temperature room with a 12 h light photoperiod. Test solutions were prepared fresh and renewed three times a week. Oxygen concentration and pH were regularly monitored, but no results were reported. The number of surviving females and number of neonates produced were recorded daily, and carapace length was measured on the last day. Various statistical treatments were used to analyse the data, and compare the effect of zineb on survival, growth and reproduction to the control animals. An EC_{50} was determined using the method described by Kooyman.

A 21-day EC_{50} of 89 µg a.i. l^{-1} (95 % confidence limits of 78 - 102 µg a.i. l^{-1}) was determined for adult survival. A LOEC of 18 µg a.i. l^{-1} was determined for effects on the intrinsic rate of natural increase, and a LOEC of >56 µg a.i. l^{-1} was determined for carapace length on day 21.

6.1.5 NEWLY SUBMITTED ZINEB DATA

In May 1999 additional data were submitted in support of the use of zineb as a booster biocide in antifouling products. These data were assessed initially, and several reference papers reviewed. However, it is considered that they would not significantly change the environmental risk assessment, so full evaluations have not been included in this evaluation (Unpublished, 1997a; Alia *et al.*, 1991).

6.2 MANCOZEB

6.2.1 ACUTE TOXICITY

6.2.2 TOXICITY TO FRESHWATER INVERTEBRATES

In 1982, a 48 h acute toxicity test was performed using mancozeb (technical grade) against *D. magna*, under static conditions. The test followed OECD Guideline 202, but was not conducted to GLP.

Four replicates of 5 daphnids (<24 h old) were exposed to nominal concentrations of 0, 0.1, 0.18, 0.32, 0.56, 1, 1.8, 3.2 or 5.6 mg mancozeb l^{-1}; the control consisted of DSW water only (groundwater with added salts). The test conditions were maintained at 20 ± 1 ^{o}C, pH 7.7 - 8.3 and dissolved oxygen concentration 6 mg l^{-1}. After 24 and 48 h the number of immobile animals, and condition of survivors was compared to the control animals.

The Author noted that the stock solutions of mancozeb were turbid, indicating that the actual concentrations of the test solutions could have been lower than the reported nominal concentrations.

The NOEC for *D. magna* was reported as nominal 0.32 mg a.i. l^{-1}. A parametric model was then used to calculate an EC_{50} for the active ingredient (see Table 6.8).

Table 6.8 Acute toxicity of mancozeb to *D. magna*

Time (h)	Nominal EC_{50} (mg a.i. l^{-1}) (95 % confidence limits)
24	1.1 (0.86 - 1.3)
48	0.66 (0.53 - 0.82)

Therefore, the 48 h EC_{50} for *D. magna* was calculated to be nominal 0.66 mg mancozeb l^{-1} (Unpublished, 1982).

In 1988, a 48 h acute toxicity test was performed using mancozeb (purity >90 %) against *D. magna*, under static conditions. The test followed US EPA FIFRA Guideline 72-2, OECD Guideline 202, and was conducted to GLP.

Duplicate groups of twenty daphnids (<24 h old) were exposed to mean measured concentrations of 0, 0.0084, 0.011, 0.015, 0.021, 0.029, 0.031, 0.035, 0.067 or 0.2 mg mancozeb l^{-1}; the control consisted of dechlorinated, aged tap water. The test conditions were maintained at 21 ± 1 ^{o}C, pH 7.7 and 8.7 - 9.3 mg oxygen l^{-1}, with a 16 h light photoperiod. The test solutions were sampled at 0 and 48 h, and actual concentrations determined by GC. After 24 and 48 h the number of immobile animals was recorded.

The hardness of the dilution medium, 350 mg $CaCO_3$ l^{-1}, exceeded the range recommended in the OECD guideline. The toxicity of zinc can decrease with increasing hardness (Hellawell, J. M., 1989), therefore, this deviation could potentially have affected the toxicity of mancozeb to *D. magna*.

The NOEC (less than 10 % immobilisation) for *D. magna* was reported to be 0.031 mg a.i. l^{-1}. Probit analysis was used to calculate an EC_{50} for the active ingredient (see Table 6.9).

Table 6.9 Acute toxicity of mancozeb to *D. magna*

Time (h)	EC_{50} (mg a.i. l^{-1}) (95 % confidence limits)
24	0.13 (0.10 - 0.18)
48	0.073 (0.063 - 0.093)

Therefore, the 48 h EC_{50} for *D. magna* was calculated to be 0.073 mg mancozeb l^{-1} (Unpublished, 1988a).

6.2.2.1 TOXICITY TO MARINE INVERTEBRATES

In 1988, a 96 h acute toxicity test using mancozeb (purity 82.4 %), in a formulation, was performed against the mysid shrimp (*Mysidopsis bahia*), under static conditions. The study was conducted to GLP, following US EPA FIFRA Guideline 72-3.

Nominal concentrations of 13, 22, 36, 60, 100 and 167 µg mancozeb l^{-1} were prepared from a stock solution in seawater (diluted to 20 ‰ salinity and filtered to 5 µm). Two replicates of 10 shrimp were exposed to each test concentration, and a seawater only control. The test conditions were kept at 19 - 23 ^{o}C, pH 7.8 - 8.2 and ^{3}2.1 - ^{3}6.5 mg oxygen l^{-1}, with a 16 h light photoperiod. The mysid shrimp were fed live brine shrimp (*Artemia salina*) throughout the test. Mortalities and sub-lethal effects were recorded daily.

Sub-lethal effects, such as lethargy and loss of equilibrium, were observed at $\geq$100 µg a.i. l^{-1} and mortality was observed at $\geq$ 60 µg a.i. l^{-1}, therefore the NOEC was reported as nominal 36 µg mancozeb l^{-1}. The LC_{50} and 95 % confidence limits were calculated using a moving average angle statistical method (see Table 6.10).

Table 6.10 Acute toxicity of mancozeb to mysid shrimp

Time (h)	Nominal LC_{50} (µg a.i. l^{-1}) (95 % confidence limits)
48	137 (115 - 174)
72	77 (65 - 91)
96	67 (58 - 79)

Therefore, the 96 h LC_{50} for the mysid shrimp was calculated to be nominal 67 µg mancozeb l^{-1} (Unpublished, 1988b).

In 1988, a 96 h acute toxicity test on shell growth in the eastern oyster (*Crassostrea virginica*), under flow-through conditions, was carried out using mancozeb (purity 82.4 %) in a formulation. The test followed US EPA FIFRA Guideline 72-3, and was performed to GLP.

Prior to the start of the test, oysters exhibiting shell growth had 2 - 5 mm of shell removed, by grinding, from their margins. Groups of twenty oysters were then each exposed to mean measured concentrations of 0, 0.71, 1.4, 2.1, 3.2 or 4 mg mancozeb l^{-1} in unfiltered seawater (salinity 30 - 35 %). The oysters were maintained at 26 ± 1 ^{o}C, pH 8.1 - 8.2 and $\geq$6.5 mg oxygen l^{-1} ($\geq$98 % saturation), with a 16 h light photoperiod. The test solutions were sampled daily and analysed by GC. After 96 h the oysters were removed from the test solutions, and shell growth measured to the nearest 0.1 mm (see Table 6.11).

Table 6.11 Shell growth in the eastern oyster

Mean measured concentration (mg a.i. l^{-1})	Mean shell deposition (mm)	Percentage reduction compared to control
Control	1.3	-
0.71	1.1	15
1.4	0.6	54*
2.1	0.5	62*
3.2	0.3	77*
4	0.3	77*

* - statistically different from the control

The significance in the difference in shell growth between the control oysters and those exposed to mancozeb was assessed using analysis of variance, and the NOEC was taken as 0.71 mg a.i. l^{-1}.

Probit analysis was used to derive a 96 h EC_{50} of 2.01 mg mancozeb l^{-1} with 95 % confidence limits of 0.33 - 12.1 mg a.i. l^{-1} (Unpublished, 1988c).

6.2.2.2 TOXICITY TO FRESHWATER FISH

In 1988, two 96 h semi-static acute toxicity tests were carried out, using technical mancozeb, against the rainbow trout and the bluegill sunfish. The studies followed OECD Guideline 203 and US EPA FIFRA Guideline 72-1, and were conducted to GLP.

Duplicates of 20 fish were each exposed to mean measured concentrations of mancozeb (see Table 6.12) in dechlorinated tap water, and a control (the nature of which was not reported). The test solutions' concentrations were determined using GC with flame-photometric detection.

Table 6.12 Mean measured concentrations of mancozeb

Mean measured concentration (mg a.i. l^{-1})	
Rainbow trout	**Bluegill sunfish**
0.044	0.026
0.074	0.043
0.135	0.073
0.283	0.095
0.496	0.2

The test conditions were maintained as shown in Table 6.13. Both species were kept in a 16 h light photoperiod. Observations were made at 3, 6 and 24 h, and then daily for mortality and signs of abnormal behaviour.

Table 6.13 Test conditions

	Rainbow trout	Bluegill sunfish
Temperature (^{o}C)	14 ± 1	21 ± 1
pH	-	7.5 - 7.7
Dissolved oxygen concentration (mg l^{-1})	-	8 - 9

- Not reported

There were several deviations from the OECD guideline during these studies. The fish were only fed up to 48 h before the start of the test, instead of up to 24 h before. This could have increased the stress on the fish, and their susceptibility to mancozeb. The water hardness was above the recommended range, at 350 mg $CaCO_3$ l^{-1}. The toxicity of zinc can decrease with increasing hardness (Hellawell, 1989), therefore, this deviation could potentially have affected the toxicity of mancozeb to rainbow trout and bluegill sunfish.

Sub-lethal effects noted in surviving fish included lethargy, being moribund, showing increased pigmentation (rainbow trout only) and lack of buoyancy (bluegill sunfish only). The NOEC, based on these sub-lethal effects, was taken as 0.044 mg a.i. l^{-1} for the rainbow trout, and 0.043 mg a.i. l^{-1} for the bluegill sunfish. The LC_{50} and 95 % confidence limits were derived from a linear model (see Table 6.14).

Table 6.14 Acute toxicity of mancozeb to freshwater fish

	LC_{50} (mg a.i. l^{-1}) (95 % confidence limits)	
Time (h)	**Rainbow trout**	**Bluegill sunfish**
48	0.15 (0.14 - 0.17)	0.094 (0.090 - 0.099)
72	0.1 (0.092 - 0.11)	0.087 (0.082 - 0.092)
96	0.07 (0.066 - 0.083)	0.083 (0.077 - 0.088)

Therefore, the 96 h LC_{50}s for the rainbow trout and bluegill sunfish were calculated to be 0.07 and 0.083 mg mancozeb l^{-1} respectively (Unpublished, 1988d & 1988e).

A 96 h study was conducted to investigate the toxicity of a formulation containing 10 % zineb and 70 % mancozeb to the cyprinid fish *Barilius bendelisis* (Deoray and Wogh, 1987). Groups of ten fish were exposed to a series of concentrations of the formulation and a tap water control, at pH 6.5, 7.5 and 9. No further details were reported. LC_{50} values were derived using probit analysis (see Table 6.15).

Table 6.15 Toxicity of a formulation containing 10 % zineb and 70 % mancozeb to *B. bendelisis*

pH	Nominal 96 h LC_{50} (mg formulation l^{-1})
6.5	0.39
7.5	0.4
9	0.42

The Authors suggested that as the acidity increased the rate of hydrolysis also increased, thus exposing the fish to metabolite(s) that were more toxic than the parent compound. However, there is actually little difference between the three LC_{50} values and no clear evidence of a pH effect on toxicity.

6.2.3 CHRONIC TOXICITY

6.2.3.1 TOXICITY TO FRESHWATER FISH

In 1994, a 28 d post-hatch chronic toxicity study was carried out with mancozeb (purity 88.07 %) against the fathead minnow (*Pimephales promelas*), under flow-through conditions. The study followed US EPA FIFRA Guideline 72-4, and was conducted to GLP.

Four replicate groups of 30 eggs (<24 h post-fertilisation) were each exposed to ^{14}C-radiolabelled mancozeb at mean measured concentrations of 0.59, 1.07, 2.19, 4.56 or 7.97 µg l^{-1}; replicate control eggs were exposed to dilution water (heat treated, filtered and UV-sterilised deep well water). The active ingredient was introduced to the aquaria using an electronically controlled dosing system. The test conditions were maintained at 24.2 - 25.8 ^{o}C, pH 7.47 - 7.93 and dissolved oxygen concentration 6.7 - 8.3 mg l^{-1}. The embryos were kept in the dark, and then once they had hatched a 16 h light photoperiod was maintained. The fry were fed live rotifers (*Brachious sp*.) and brine shrimp nauplii (*Artemia*). The actual concentrations of the test solutions were measured throughout the test using GC.

The developing embryos were observed daily for mortality. The post-hatch growth period began after 8 d; then the fry were observed daily for abnormal behaviour, physical change and mortality. Fry growth was determined on day 28 by measuring weight and length. Data were statistically analysed using contingency tables (discrete data, e.g. survival) or analysis of variance (continuous data, e.g. length). Spinal curvature and loss of buoyancy were observed in several individuals at 4.56 and 7.97 µg a.i. l^{-1}. For all results see Table 6.16.

Table 6.16 Chronic toxicity of mancozeb to fathead minnow

Mean measured concentration (µg a.i. l^{-1})	Hatching success (%)	Percent survival	Mean standard length (mm)	Mean blotted wet weight (g)
Control	84.9	87.1	23.3	0.211
0.592	87.5	94.3	23	0.195
1.07	90.0	93.5	23.3	0.206
2.19	93.4	91.1	23.1	0.199
4.56	89.2	62.6*	22.1**	0.184**
7.97	92.4	22.9*	19**	0.131**

* - Significantly lower than the control

** - Groups excluded from the statistical analysis due to significant survival effects

The most sensitive biological endpoint was survival, therefore, the 28 d LOEC was 4.56 µg a.i. l^{-1} and the NOEC 2.19 µg a.i. l^{-1}. The geometric mean of these two values is the maximum acceptable toxicant concentration (MATC), and was calculated to be 3.16 µg a.i. l^{-1} (Unpublished, 1994).

6.3 METABOLITES

6.3.1 GROWTH INHIBITION OF FRESHWATER ALGAE

A study was conducted by van Leeuwen, *et al.,* (1985a) to investigate the acute toxicity of DIDT (purity 98 %) and ETU (purity 99 %) to the freshwater algae *C. pyrenoidosa*. The methodology was as described in Section 6.1.2. The most sensitive endpoint for DIDT was growth; a 96 h EC_{50} of 0.18 mg l^{-1} was derived. The most sensitive endpoint for ETU was yield with a 96 h EC_{50} of 860 mg l^{-1}.

6.3.2 ACUTE TOXICITY

6.3.2.1 TOXICITY TO FRESHWATER INVERTEBRATES

A study was conducted by van Leeuwen, *et al.,* (1985a) to investigate the acute toxicity of DIDT (purity 98 %) and ETU (purity 99 %) to *D. magna*. The methodology was as described in Section 6.1.3.1. A 48 h EC_{50} of 0.21 mg l^{-1} (95 % confidence limits of 0.18 -0.24 mg l^{-1}) was derived using the Litchfield and Wilcoxon method for DIDT. A 48 h EC_{50} of 26.4 mg l^{-1} (95 % confidence limits of 21.6 - 32.2 mg l^{-1}) was similarly derived for ETU.

6.3.2.2 TOXICITY TO FRESHWATER FISH

A study was conducted by van Leeuwen, C. J. *et al* (1985a) to investigate the acute toxicity of DIDT (purity 98 %) and ETU (purity 99 %) to the guppy. The methodology was as described in Section 6.1.3.3. A 96 h LC_{50} of 0.49 mg l^{-1} (95 % confidence limits of 0.32 - 1 mg l^{-1}) was derived using the Litchfield and Wilcoxon method for DIDT. A 96 h LC_{50} of 7500 mg l^{-1} (95 % confidence limits of 5600 - 10,000 mg l^{-1}) was similarly derived for ETU.

6.3.3 CHRONIC TOXICITY

6.3.3.1 TOXICITY TO FRESHWATER INVERTEBRATES

A study was conducted by van Leeuwen, *et al.,* (1985b) to investigate the effect of DIDT (purity 98 %) and ETU (purity 99 %) on survival, reproduction and growth in *D. magna*. The methodology was as described in Section 6.1.4.1. Twenty-one day EC_{50}s of 73 µg DIDT l^{-1} (95 % confidence limits of 67 - 81 µg l^{-1}) and 18,000 µg ETU l^{-1} (10,000 - 32,000 µg ETU l^{-1}) were determined for adult survival. LOECs of 56 µg DIDT l^{-1} and $\leq$1000 µg ETU l^{-1} were determined for effects on the intrinsic rate of natural increase. LOECs of 56 µg DIDT l^{-1} and 10,000 µg ETU l^{-1} were determined for carapace length on day 21.

6.4 SUMMARY OF ECOTOXICOLOGY DATA

6.4.1 ZINEB

Most of the submitted company studies were performed to GLP, however none followed a recognised guideline. Some of the literature studies were, however, performed to OECD guidelines. The ecotoxicology of zineb can be summarised as follows.

A published paper highlighted the toxicity of zineb to soil micro-organisms involved in nitrification, with EC_{50} values of 3.6 and 16 µg a.i. g^{-1} dry weight of soil and NOECs of 0.4 and 2.5 µg a.i. g^{-1} dry weight of soil depending on the soil type. This indicated a potential for zineb to be toxic to micro-organisms in sewage treatment works, and also those living in aquatic ecosystems.

Several studies were evaluated that investigated the toxicity of zineb to freshwater and marine organisms (see Tables 6.17 and 6.18). Unfortunately, most zineb endpoints were derived from nominal exposure concentrations, and zineb was shown to be hydrolytically unstable, therefore, it is possible that the true toxicity has been underestimated. It is also possible that the use of static test solutions led to the exposure of the test species to one or more toxic metabolites rather than the parent compound. The most acutely sensitive species to zineb was *D. magna* with a nominal 48 h LC_{50} of 0.97 mg l^{-1}.

Chronic data were only obtained for one species (*D. magna*), therefore an assessment of the most sensitive aquatic species could not be made.

Two robust studies indicated that zineb was of low toxicity to birds, with an acute oral NOEL of nominal >2000 mg a.i. kg^{-1} (bobwhite quail), and acute dietary NOECs of nominal >5000 ppm (bobwhite quail and mallard duck).

Table 6.17 Acute nominal toxicity of zineb to aquatic organisms

Species	Water type	GLP	Test system	Endpoints (mg a.i. l^{-1})
Algae (*C. pyrenoidosa*)	Fresh	**X**	Static	96 h EC_{50} (inoculum) = 1.8
D. magna	Fresh	3	Static	48 h EC_{50} = 1.4
		X	Semi-static	48 h EC_{50} = 0.97*
Brown shrimp (*C. crangon*)	Salt	3	Semi-static	96 h LC_{50} = >200
Rainbow trout (*O. mykiss*)	Fresh	**X**	Static	96 h LC_{50} = 29
		3	Static	96 h LC_{50} = 42
Bluegill sunfish (*L. macrochirus*)	Fresh	3	Static	96 h LC_{50} = 35
Guppy (*P. reticulata*)	Fresh	**X**	Semi-static	96 h LC_{50} = 7.2**
Juvenile plaice (*P. platessa*)	Salt	3	Static	96 h LC_{50} = 26 - 40

* OECD guideline 202 ** OECD guideline 203

Table 6.18 Chronic nominal toxicity of zineb to freshwater aquatic organisms

Species	Water type	GLP	Test system	Endpoints (mg a.i. l^{-1})
D. magna	Fresh	**X**	Semi-static	21 d EC_{50} = 0.089 LOEC (IRNI) = 0.018 LOEC (carapace length at 21 d) = >0.056

IRNI = intrinsic rate of natural increase

6.4.2 MANCOZEB

Most of the submitted company studies were performed to GLP, and all followed OECD and/or US EPA guidelines. The ecotoxicology of mancozeb (as a model for zineb) can be summarised as follows.

Several studies were submitted that investigated the toxicity of mancozeb to freshwater and marine invertebrates and fish (see Table 6.19). The mysid shrimp was the most sensitive species with a 96 h LC_{50} of 0.067 mg a.i. l^{-1}.

Table 6.19 Toxicity of mancozeb to aquatic organisms

Species	Water type	Test	Guideline	GLP	Test system	Endpoint (mg a.i. l^{-1})
D. magna	Fresh	Acute	OECD 202	**X**	Static	Nominal 48 h EC_{50} = 0.66 NOEC = 0.32
			OECD 202 US EPA FIFRA 72-2	3	Static	48 h EC_{50} = 0.073 NOEC = 0.031
Mysid shrimp (*M. bahia*)	Salt	Acute	US EPA FIFRA 72-3	3	Static	Nominal 96 h LC_{50} = 0.067 NOEC = 0.036
Eastern oyster (*C. virginica*)	Salt	Acute	US EPA FIFRA 72-3	3	Flow-through	96 h EC_{50} (shell deposition) = 2.01 NOEC = 0.71
Rainbow trout (*O. mykiss*)	Fresh	Acute	OECD 203 US EPA FIFRA 72-1	3	Semi-static	96 h LC_{50} = 0.07 NOEC = 0.044
Bluegill sunfish (*L. macrochirus*)	Fresh	Acute	OECD 203 US EPA FIFRA 72-1	3	Semi-static	96 h LC_{50} = 0.08 NOEC = 0.043
Cyprinid species (*B. bendelisis*)	Fresh	Acute	-	X	Assumed static	96 h LC_{50} = 0.4 (formulation containing 70 % a.i.)
Fathead minnow (*P. promelas*)	Fresh	Chronic	US EPA FIFRA 72-4	3	Flow-through	28 d LOEC (survival) = 0.00456 NOEC (survival) = 0.00219

6.4.3 METABOLITES

A published paper was evaluated which reported acute and chronic toxicity endpoints for several metabolites of zineb (see Tables 6.20 and 6.21). Most of the data were generated as required by OECD guidelines. The ecotoxicology of the metabolites of zineb can be summarised as follows.

The most acutely sensitive species to the metabolites were the algae, with a 96 h EC_{50} (growth) of 0.18 mg DIDT l^{-1} and *D. magna* with a 48 h EC_{50} of 26.4 mg ETU l^{-1}. DIDT appeared to have similar levels of toxicity to aquatic organisms as zineb. ETU was considerably less toxic than zineb or DIDT to algae and fish, and slightly less toxic to *Daphnia*.

Chronic data were only obtained for one species (*D. magna*), therefore an assessment of the most sensitive aquatic species could not be made.

Table 6.19 Acute nominal toxicity of zineb metabolites to freshwater aquatic organisms

Species	Metabolites	Test system	Endpoints (mg a.i. l^{-1})
Algae (*C. pyrenoidosa*)	DIDT	Static	96 h EC_{50} (growth) = 0.18
	ETU	Static	96 h EC_{50} (yield) = 860
D. magna	DIDT	Semi-static	48 h EC_{50} = 0.21*
	ETU	Semi-static	48 h EC_{50} = 26.4*
Guppy (*P. reticulata*)	DIDT	Semi-static	96 h LC_{50} = 0.49**
	ETU	Semi-static	96 h LC_{50} = 7500**

* OECD guideline 202
** OECD guideline 203

Table 6.20 Chronic nominal toxicity of zineb metabolites to freshwater aquatic organisms

Species	Metabolites	Test system	Endpoints (mg a.i. l^{-1})
D. magna	DIDT	Semi-static	21 d EC_{50} = 0.073 LOEC (IRNI) = 0.056 LOEC (carapace length at 21 d) = 0.056
	ETU	Semi-static	21 d EC_{50} = 18 LOEC (IRNI) = $\leq$1 LOEC (carapace length at 21d) = 10

IRNI = intrinsic rate of natural increase

6.5 ASSESSMENT OF READ-ACROSS ARGUMENT

6.5.1 INTRODUCTION

Ecotoxicological hazard data in support of the use of zineb as an antifouling booster biocide has been submitted. A reasoned argument that stated that the structure and properties of mancozeb were similar enough to justify read-across between the two chemicals when assessing environmental hazard data was also submitted.

6.5.2 CONSIDERATION OF THE READ-ACROSS ARGUMENT

It was accepted that zineb and mancozeb have extremely similar structures, both being co-polymeric compounds comprising of chains of $EBDC^{2-}$ ions and metal ions (zinc in zineb, and zinc and manganese in a ratio of 10:1 in mancozeb).

Endpoints for acute toxicity to freshwater species of algae, invertebrates and fish were very similar for zineb and mancozeb. Unfortunately no data were presented for the chronic toxicity of mancozeb to aquatic organisms, so this level of effect could not be compared. Various papers referenced in the read-across argument suggested that the metabolites of zineb and mancozeb made a greater contribution to their toxicity than the parent compound.

Considering the rapid rate at which both compounds decompose in water, this suggestion was probably correct.

In conclusion, the argument that zineb and mancozeb were similar enough in structure and properties to support approval of use of zineb in antifouling products (AFPs) with mancozeb data was accepted.

7 ENVIRONMENTAL RISK ASSESSMENT

7.1 ENVIRONMENTAL HAZARD PROFILE

The leaching rate for zineb, from a number of different sources, varied from 0.04 - >10 µg cm^{-2} d^{-1} for paints with varying a.i. content. Zineb was shown to hydrolyse, and a half-life of 16.89 d reported at pH 7.96 (which was considered more applicable than pH 7 with respect to the marine environment). The route of aquatic degradation appeared to be affected by the presence/absence of copper ions. In the absence of copper zineb degraded to the major metabolites DIDT and ETU. In the presence of copper degradation to DIDT still occurred, with subsequent degradation to the major metabolite EDI. Little information was provided on the persistence of these metabolites. The degradation of zineb in water-sediment systems was not investigated. The adsorption and degradation of zineb in soil was not assessed. No bioconcentration data were provided, however, with a log Pow of 0.77 there is little potential for zineb to bioconcentrate in aquatic organisms. The species most sensitive to chronic effects was *D. magna* with a 21 d LOEC (intrinsic rate of natural increase) of 18 µg a.i. l^{-1}.

7.2 RISK ASSESSMENT STRATEGY

The risk assessment has been concentrated on the marine environment since the data available are predominantly for the use of antifouling products (AFP's) in estuarine and coastal areas, although the risk to freshwater environments has not been precluded. However, the strategy for assessing risk to the marine environment is less well developed than for terrestrial or freshwater. Therefore, the risk assessment strategy adopted for evaluation purposes has been presented in a separate document 'Environmental Risk Assessment of Booster Biocides in Antifouling Products' (ACP 2002). This document presents a comprehensive and comparative risk assessment for all approved booster biocides and has been endorsed by an expert *Ad-hoc* Environmental Panel.

For the marine environment, three distinct areas were identified; estuarine (including marinas and harbours), shallow coastal seas and deep ocean. Within each area considerations of the following compartments; sediment (including suspended sediment), water and associated biota are required. Therefore the primary objective of this risk assessment has been to establish the likelihood of zineb reaching (or having reached) a concentration in the aquatic environment that would adversely affect some component of that environment. In order to achieve this objective predicted environmental concentrations (PECs) and predicted no effect levels (PNECs) have been derived.

Below are the main points of the risk assessment detailed in 'Environmental Risk Assessment of Booster Biocides in Antifouling Products' (ACP 2002) concerned with the use of antifoulants containing zineb, however, reference to the full evaluation is advised.

7.2.1 ENVIRONMENTAL CONCENTRATIONS

The direct and indirect exposure of the aquatic environment to zineb as a result of AFP (antifouling product) use are detailed fully in Section 3.2 of 'Environmental Risk Assessment of Booster Biocides in Antifouling Products' (ACP 2002). Data have not been made available to address the direct and indirect inputs to the soil, and aquatic compartments as a

result of AFP's use (application and removal stages). Further to this, HSE have no data regarding the direct exposure of STP (sewage treatment processes), or the subsequent emissions to surface waters. However, a survey carried out by the EA (Environment Agency, 1998) demonstrated that there was a wide range of use patterns and work practices regarding the application and removal of AFPs. The survey suggested that emissions via drains would be low, since direct exposure of the surrounding water body (marina/harbour) was more likely, with the majority of boat maintenance taking place at the waters' edge. Although the survey did state that where removal by sandblasting was undertaken (usually by boatyards) appropriate waste disposal methods were reported. The contamination of aquatic sediments from direct emissions or via particulate inputs (contaminated soil) is an important route to consider, since the potential to contribute significantly to the environmental loading of the booster biocides exists through remobilisation. Where strongly adsorbed a.i.s are demonstrated, in the aquatic environment mechanical remobilisation, or sediment redistribution via dredging are likely. Therefore, exposure scenarios other than direct exposure of the aquatic compartment (i.e. indirect exposure of the aquatic environment, direct exposure of the soil and STP) have not been considered further at this stage with insufficient data available to fully address the risks. The potential scale of direct exposure to the aquatic compartment may also be considered to outweigh the additional areas of concern at this time.

Usage information was requested from all the current approval holders for zineb, however, the information received was too limited to allow for any accurate assessment of the actual amounts used of AFP's containing zineb. Therefore, the results of a survey conducted by the EA (Environment Agency, 1998) were used instead. The EA survey demonstrated that zineb was not significantly used in AFP's for pleasure crafts in the UK (< 3 %). No measured data are available for zineb. However, the current monitoring data could only ever represent the usage levels for 1998, and the Ad-hoc Environmental Panel considered predictions of maximum PECs necessary since post approval usage cannot be controlled. Therefore, PEC data based on 100 % usage (all vessels treated) of zineb AFP's were predicted using a model developed as part of a HSE/EA commissioned research project. The model, REMA (**R**egulatory **E**nvironmental **M**odelling of **A**ntifoulants), is a steady-state QWASI (quantitative water air sediment interface) model, designed to predict concentrations of biocides in both the water and sediment compartments of estuaries/marinas/harbours. The model is based upon four real estuary scenarios in the UK, for which the model has been successfully validated. The model inputs used for zineb are presented in Table 7.1, and a summary of the results in Table 7.2.

Table 7. 1: REMA model input parameters for zineb

Parameter	Value	Source
Chemical type (organic/involatile)		
Melting point	157 ºC	Company
Molecular mass	275.8	Company
Solubility	0.07 mg l^{-1}	Company
Vapour pressure	3.98 x 10^{-4} Pa	Company
Log Koc	0.38	Calculated from Kow
Sediment reaction half-life (h) Hydrolysis Photolysis Biodegradation	 10 000 000 10 000 000 10 000 000	 Default (no data) Default (no data) Default (no data)
Water reaction half-life (h) Hydrolysis Photolysis Biodegradation	 405.3 10 000 000 10 000 000	 Company, hydrolysis (pH 7.96) Default (no data) Default (no data)
Leaching rate from vessel surface (mg cm^{-2} d^{-1})	10	Company data, maximum
Proportion of leisure craft treated	3.0 % and 100 %	EA 1998 Survey

Table 7.2: Summary of PEC calculations for zineb in estuaries and open marinas

Usage (%)	Site	PEC_{water} (ng a.i. l^{-1})	$PEC_{sediment}$ (µg a.i. g^{-1})
3	Estuary*	68.0 (± 111.5)	$3.3x10^{-6}$ ($\pm$ $5.4x10^{-6}$)
	Open marina**	197.5 (± 118.9)	$2.4x10^{-5}$ ($\pm$ $1.4x10^{-5}$)
100	Estuary*	2266.5 (± 3715.2)	$1.1x10^{-4}$ ($\pm$ $1.8x10^{-4}$)
	Open marina**	6583.0 (± 3963.4)	$7.9x10^{-4}$ ($\pm$ $4.8x10^{-4}$)

* n = 12, **n = 7

The mean PEC data for zineb presented above exceeded the highest limit of detection quoted by CEFAS (> 5 ng l^{-1} for zinc pyrithione) in all cases. However, the mean PEC calculations for the sediment compartment were very low in all cases. Therefore, since no limit of detection has been made available it is not known at present whether the levels presented in Table 7.2 could be detected (no analytical technique was developed for zineb).

7.2.2 PREDICTED NO EFFECT CONCENTRATIONS

Once released into the aquatic compartment, the chemical fate of the booster biocide will determine whether the toxic effect exerted is limited to the target organisms within a boundary layer of a painted surface or whether the a.i. persists and there is potential for exposure to non-target organisms. Therefore, selection of key non-target organisms and likely duration of exposure is essential, but this is somewhat reliant on the availability of acceptable data for representative marine species. Chronic data endpoints have been selected as more appropriate for the purpose of a marine risk assessment following the use of booster biocides. This is because the inputs of booster biocides into the marine environment as a result of leaching from multiple point sources (treated surfaces) will be a continuous process.

Even where high degradation is indicated from fate studies, the continuous input from leaching can still result in long-term exposure. This will only be mitigated if the leaching rate of an a.i. from a painted surface is significantly slower than the rate of degradation, and no toxic metabolites are produced.

Comparisons between marine and freshwater chronic toxicity data for booster biocides have not demonstrated any differences in sensitivities. Therefore, in the absence of chronic marine data, freshwater data would be acceptable. Further to this, in considering the number and quality of tests available for the current review, the most sensitive species has been selected, regardless of test medium. However, the introduction of safety factors is required before deriving a PNEC from the available hazard data, which in the absence of additional or more appropriate data will provide a suitable safety margin for all marine organisms. The provision of safety factors has been made in accordance with the guidance detailed in the European Risk Assessment Technical Guidance Document (EURATGD, 1996), and those previously accepted by the Committees (see Appendix 3).

The most sensitive species to chronic zineb exposure was shown to be *D. magna* (published study), in the absence of suitable marine data. No company-submitted chronic data were available, therefore, in light of the limited available data a 1000 safety factor was applied, and a PNEC of 0.018 mg a.i. l^{-1} given based on a 21 d LOEC (no details given) for *D. magna*.

7.2.3 RISK QUOTIENT

The PEC:PNEC calculations derived from the predicted data based on 100 % usage for zineb were considered to be the most appropriate for regulation. This approach was suggested and endorsed by the *Ad-hoc* Environmental Panel, for the primary reason that post-approval control of amounts used is not possible, and therefore a worst-case assumption is necessary. In addition to this the only usage data currently available are those regarding the number of pleasure crafts located in UK waters, therefore, the inputs from ships would be additional. Finally, no allowance has been be made regarding the potential for additivity, synergism or antagonism between a.i.s which will coexist in the aquatic environment as a result of leaching from AFP's. This was considered as additional support for the 100 % 'worst case' usage approach.

Table 7.3 Summary of zineb PEC: PNEC ratios based on low (3 %) and maximum (100 %) use on pleasure crafts

% USE	Estuary PEC:PNEC		Open marina PEC:PNEC	
	Mean (± STD)	% Exceedence *	Mean (± STD)	% Exceedence *
3	3.8 (± 6.2)	58.3	11.0 (± 6.6)	100
100	125.9 (± 206.4)	100	365.7 (± 220.2)	100

* - % Exceedence is the percentage of single samples which were shown to exceed the MEC:PNEC quotient of 1, demonstrating that the measured concentration of booster biocides was greater than the 'no effect concentration' and therefore indicating significant risk of adverse effects.

The predicted data for zineb at both low (3 %) and maximum (100 %) usage levels resulted in unacceptable PEC:PNEC ratios for both estuary and open marina sites (see Table 7.3).

However, consideration of the input data for zineb has highlighted three major areas of insufficient data;

i) Degradation rates for zineb (hydrolysis only, based on very limited study)

ii) Leaching rate (reported range from non-standard tests, therefore, highest of > 10 mg cm^{-2} d^{-1} used), and

iii) High safety factor selection, due to the paucity and low quality ecotoxicological data available.

7.3 SIGNIFICANCE OF RESULTS

7.3.1 WATER CONCENTRATIONS

The predicted data provided by the REMA model have allowed development of the risk assessment to take on board the maximum risk posed from the pleasure craft use of zineb AFP's in the UK. Unfortunately it has not been possible to predict the environmental concentrations resulting from the use of zineb on larger commercial vessels and ships. Therefore, the predicted concentrations at the 100 % use level (for pleasure crafts) are more likely to underestimate the environmental exposure resulting from AFP's use.

The exceedences predicted for zineb using the REMA model data indicated that refinement of the current risk assessment is necessary since unacceptable levels have been predicted for both estuary areas and open marinas using 100 % usage on leisure crafts. However, because of the present level of data submitted to support this a.i., no conclusion can be reached regarding the risk to the aquatic environment from zineb or any subsequent metabolites. However, with additional data to support the rapid degradation of zineb and clarification as to the routes of metabolism in the aquatic environment, the risk assessment can be refined.

7.3.2 SEDIMENT TOXICITY

The $PEC_{sediment}$ data derived by the REMA model suggest that the sediment compartment was not a concern for zineb. Therefore, no additional data to address zineb's behaviour or toxicity in sediment have been requested.

7.3.3 SECONDARY AND SUB-LETHAL ECOTOXICOLOGY DATA

Potential secondary effects as a result of fish bioconcentration of zineb have not been addressed. The potential for indirect or sub-lethal effects (i.e. endocrine disruption), should be considered in all cases where prolonged exposure to concentrations below those shown to elicit known toxic effects are likely. This consideration should remain until reliable data can be obtained which demonstrates that such effects do not occur. This is especially important when considering the use of booster biocides that result in direct environmental exposure. Published data have indicated that there is a potential for zineb to exert endocrine disruption in higher organisms. However, whilst the *Ad-hoc* Environmental Panel recognised that the significance of these data are difficult to determine, since assessments for endocrine disruption (and other sub-lethal effects) are not yet available, they recommended that the potential for both these effects should not be ignored. Indeed, when the techniques become

available the panel members agreed that persistent a.i.s used in AFP's (or their metabolites) should be reconsidered.

7.3.4 METABOLITE FATE AND EFFECTS DATA

Degradation of a parent compound is often where the considerations for environmental risk end. However, risk assessments should be conducted for all reported persistent major (> 10 %) metabolites. Without consideration of the metabolites, a full assessment of the risk to the marine environment from the use of AFPs cannot be considered complete. Clarification has been requested for the metabolism of zineb in marine sediment-water systems, and where significant persistent metabolites are highlighted additional data will be requested.

7.3.5 MIXTURES OF SUBSTANCES

AFPs usually contain a mixture of booster biocides with copper or TBT compounds. Therefore, the environment will be routinely exposed to such mixtures as a result of *in situ* leaching. Under the current regulations (COPR, 1986) AFPs are registered and approved on an a.i. basis, and mixtures of a.i.s are not considered. General understanding of the possible effects arising from mixtures such as synergism, additivity and antagonism, is as yet not sufficiently developed. Also, when considering the number of AFPs and potential combinations of these in the field at any one time, the applicability of laboratory studies in gaining an understanding of effects in the field becomes so complex that a realistic assessment is unlikely. In addition to this, the chemistry of booster biocides in paint matrices is very complicated and caution would have to be applied in the interpretation of results.

A simpler approach may be to consider the mode of action of the a.i.s present in AFPs, for example zineb and other a.i.s with known fungicidal action. However, in the absence of data, mixtures will not be considered further within this risk assessment with the assumption that assuming 100 % use of single a.i.s takes at the very least additivity into account.

7.4 DATA REQUIREMENTS

Additional data requirements have been identified below as essential for the refinement of the current risk assessment;

1. Leaching rate of the parent a.i. from a treated surface using representative products to be submitted within 12 months.

2. Confirmation of aquatic metabolism of zineb in marine sediment-water systems, including full characterisation of the metabolites (addressing effects of copper ions) to be submitted within 12 months.

3. Chronic exposure of the parent compound to a suitable marine species to be submitted within a further 12 months of data requirements 1 and 2 (significant reductions in the leaching rate and/or degradation rate may reduce the predicted concentrations, negating the need to reduce the current safety factor).

4. Once the fate of zineb has been established and the significance and persistence of the metabolites is known, additional ecotoxicology, fate and behaviour data may be required.

Additional proposals pertinent to the overall environmental risk assessment of booster biocides have been made in 'Environmental Risk Assessment of Booster Biocides in Antifouling Products' (ACP 2002).

8 EFFICACY

Efficacy data on antifouling products containing zineb were not included in the partial review presented to the ACP in 1999 and have not previously been evaluated by the Committees.

8.1 BACKGROUND/INTRODUCTION

Two processes describing the fouling of an immersed surface by organisms are recognised in the literature, and are presented in 2 papers.

The first of these papers describes a classical view of fouling, in which fouling is reported to occur as a successional process. A freshly immersed surface rapidly adsorbs various organic molecules to form a conditioning film. This film facilitates subsequent colonisation by such micro-organisms as bacteria and diatoms, which results in the formation of a slime layer. The microfouling slime layer is subsequently succeeded by macrofouling. This can be of two types; 'hard' and 'soft' fouling. Common soft-fouling organisms include algae, sponges, tunicates and hydroids. Common hard-fouling organisms include barnacles, mussels, clams and tubeworms. Larvae of fouling organisms are more diverse and numerous in coastal areas than in the open sea, therefore the fouling challenge is more intense in these areas (US Naval Institute, 1981).

Other workers have offered an alternative theory to succession. This is a much more complex process depending on the relative amount and type of fouling organism present. The different organisms are in dynamic equilibrium with the immersed surface, and in its absence, with flocculation of 'marine snow' (waste material, dead micro-organisms etc falling through the water column). There are a number of secondary driving forces and behavioural interactions in this model. For example, certain types of microbial fouling may actually inhibit the settlement of macrofouling organisms (Clare *et al*., 1992).

Animal and weed fouling of vessel surfaces increases the drag on the hull. This results in increased fuel costs and a reduction in the vessel's speed and manoeuvrability. Beyond a certain level, fouling will require removal from the vessel by its owner during dry-docking. The performance of antifouling products may be judged as the maximum specified time that a vessel can spend 'in service' before having to be dry-docked, cleaned and recoated. For insurance purposes, commercial vessels have to be dry docked at least every 5 years for inspection and maintenance. Therefore, the ideal antifouling product would protect a vessel for this length of time, so that extra expense is not incurred as a result of more frequent dry-docking. The period of time between repainting can, however, vary quite considerably depending upon the chosen antifouling product formulation, and the environment in which it is used. Various environments will present different fouling challenges.

Parameters which will influence the service time of an antifouling product include:-

Trading patterns (coastal or deep sea, turn around time, speed of vessel etc.),
Fouling conditions (warm or temperate water),
Physico-chemical conditions of the sea water e.g. pH and temperature,
Coating type and film thickness.

8.2 ACTIVE SUBSTANCES/COATING TYPES USED IN ANTIFOULING PRODUCTS

Antifouling products can be broadly divided into two types: Those that contain tributyltin (TBT), and those that are TBT-free.

TBT-free products typically contain copper or a copper compound such as copper (I) oxide (Cu_2O), or copper (I) thiocyanate (CuSCN) as the principal biocide. Since some of the common algae such as *Enteromorpha* spp. and *Amphora* spp. are tolerant of copper, this active ingredient is 'boosted' or enhanced by the presence of one or more organic biocides such as Irgarol 1051 or dichlorophenyl dimethylurea (Diuron). These are usually algicides, but may, in addition, possess a wider spectrum of antifouling activity.

Copper is also used to enhance the performance of TBT products because organisms such as *Ectocarpus* spp. and *Achanthes* spp. are tolerant of tributyltin oxide (TBTO) (Callow, 1990; Unpublished, 1994).

The antifouling products can be further categorised into the following broad coating types:

Soluble matrix (formerly known as conventional),
Insoluble matrix (formerly known as contact leaching),
TBT and TBT-free self-polishing copolymer (formerly known as TBT and TBT-free ablative).

The categorisation of coating types outlined above is very generalised. It should be noted that apart from the TBT self-polishing co-polymer (SPC) products, the majority of other antifouling products do not necessarily rely on one single coating technology. Instead, composites of different technologies have been developed by antifouling formulators to suit customer specifications and environmental requirements.

Further detail and descriptors for the individual coating types can be found in Appendix 4.1.

The Antifouling Manufacturers Working Group of the European Council of Paint, Printing Ink and Artists' Colours Industry (CEPE) have agreed maximum protection periods that can be expected for each antifouling coating type. The types of coating and maximum protection periods with respect to recommended dry docking intervals are summarised in Table 8.1.

Table 8.1 Maximum periods of service for various types of antifouling product

Type of formulation	Conventional	Contact leaching	TBT free ablative	TBT ablative (SPC)
Maximum period of service	18 months	24 months	3 years	5 years

It should be noted that the maximum protection periods presented in Table 8.1 are a generalisation of maximum protection periods that may be achieved within these very broad groupings. In reality, these agreed intervals reflect a compromise position reached between CEPE members. In addition Table 8.1 does not provide an indication of the level of performance that can be obtained by a product specified within those time periods, or the

level of performance required by a particular specification. Performance ratings are heavily dependent upon the particular coating being applied to specification (surface preparation, primers, undercoatings, dry film thickness etc.), trading and sailing pattern of the vessel and a wide variety of environmental factors.

8.3 CURRENTLY APPROVED ANTIFOULING PRODUCTS CONTAINING ZINEB

There are currently (November 1999) 32 approved antifouling products that contain zineb. Six Approval Holders hold these 32 product approvals. The distribution of zineb antifouling products by active ingredient is shown in Table 8.2.

Table 8.2 The distribution of zineb antifouling products by active ingredient

ACTIVE INGREDIENT(S)	No.	ACTIVE INGREDIENT(S)	No.
Zineb/Cu_2O	4	Zineb/Cu_2O/TBTM/TBTO	17
Zineb/Cu_2O/copper resinate	1	Zineb/CuSCN/TBTM	2
Zineb/Cu_2O/TBTM	6	Zineb/CuSCN/TBTM/TBTO	2

Information on the coating type of each product has been received for 31 of the 32 products that contain zineb. Although the products may often be formulated as a composite of different coating technologies, Approval Holders have categorised their formulations according to the coating types that ***best*** describes them. In addition, most Approval Holders have provided information on maximum dry-docking intervals for their products. Coating types and dry docking intervals are presented in Table 8.3.

Table 8.3 Coating types and dry docking intervals for products containing zineb

Coating type	No of products having maximum recommended dry-docking intervals				
	12 mths	24 mths	36 mths	60 mths	Unspecified
Ablative (TBT)	1	4	-	20	-
Ablative (TBT-free)	-	1	2	-	-
Contact leaching	-	2	-	1	-
Unspecified	-	-	-		1

Table 8.3 shows that 1 contact leaching coating type product exceeds CEPE agreed maximum dry-docking periods (see Table 8.1). 'Unspecified' refers to a product where the Approval Holder has not provided information.

Concentration ranges of biocides in zineb products are presented in Table 8.4.

Table 8.4 Concentration ranges of biocides in zineb products

Coating type	Biocides (and range as appropriate) % w/w						No of products
	Cu_2O	CuSCN	Zineb	TBTM	TBTO	Copper resinate	
Ablative (TBT)	0 - 50	0 - 30	0 - 18	10 - 30	0 - 1	-	25
Ablative (TBT-free)	10 - 65	-	0.25 - 10	-	-	-	3
Contact leaching	5 - 50	-	0.25 - 15	0 - 15	0 - 0.8	0 - 2.45	3
Unspecified	4	-	4.45	19.81 - 20.35	-	-	1

Table 8.4 shows that the level of zineb does not exceed 18 % w/w.

8.4 EFFICACY DATA SUBMITTED IN SUPPORT OF THE REVIEW OF THE USE OF ZINEB IN ANTIFOULING PRODUCTS

To date, unpublished data and a technical literature search have been included in Section 8.4.1 (simulated use tests).

These data have been evaluated by HSE to illustrate that the use of zineb (in combination with copper as principal biocide, or with other organic biocides) will result in antifouling products that demonstrate effective antifouling capability.

8.4.1 SIMULATED USE TESTS

8.4.1.1 INTRODUCTION TO RAFT TESTING

Due to the accessibility of test sites (most companies have access to raft testing facilities) and ease of operation, raft testing has been widely adopted for antifouling trials. As such, they are important indicators of the antifouling capability of biocidal active substances.

They are static tests conducted usually with submerged panels coated with the test formulation. These tests are generally used to demonstrate the effectiveness of an antifouling product relative to an uncoated (blank) substrate. The method is unable to evaluate complete coating systems (especially the more advanced SPC or ablative technologies) or the relative lifetime of coatings. This means that raft testing is unable to predict the actual performance in service. Raft tests can, however, simulate the use of a product on a yacht, which will spend most of its time stationary (anchored/berthed) (Unpublished, 1996d).

Efficacy test data generated in this way have been provided by Approval Holders. Studies have been conducted on a range of products. These products are representative of current approved formulations that contain zineb, both in terms of levels of zineb present in combination with copper and TBT, and the coating type. Both the levels of zineb present, in

combination with copper and/or TBT as the principal biocide, and the coating types have been encompassed.

The studies, unless otherwise stated, were generated in accordance with the CEPE Antifouling Working Group Method of the Generation of Efficacy Data (Unpublished, 1993a - see Appendix 4.2). In this method, results are presented using scoring and assessment systems devised by the individual companies or test houses (NB there is no current uniform industry standard for scoring/presentation of data).

8.4.1.2 TBT-FREE SELF POLISHING COATING TYPE

A raft test was conducted at 3 test sites according to a standard company test method described in Appendix 4.3, Section 2. Sites 1 and 2 were located in the UK with site 3 in Singapore. The test product was a duplex paint system comprising a slow polishing base coat overcoated with a faster polishing topcoat. The system was designed for deep sea and coastal use, and comprised of Cu_2O and zineb as the active ingredients. The base coat could be used independently of the topcoat if required. The topcoat was not recommended for use in isolation.

Two coats of a dry film thickness of 150 µm were applied by brush to a marine plywood test panel measuring approximately 600 x 600 mm. The two coats consisted of either base coat plus base coat, or base coat plus topcoat. The actual test area of the panel was 500 x 90 mm. No base coat only panels were tested at site 3.

The panels were immersed for 19 months at site 1 (October 1994 to May 1996), for 19 months at site 2 (September 1994 to April 1996) and for 16 months in at site 3 (January 1995 to May 1996).

The results of the study are presented in Figures 8.1 to 8.3.

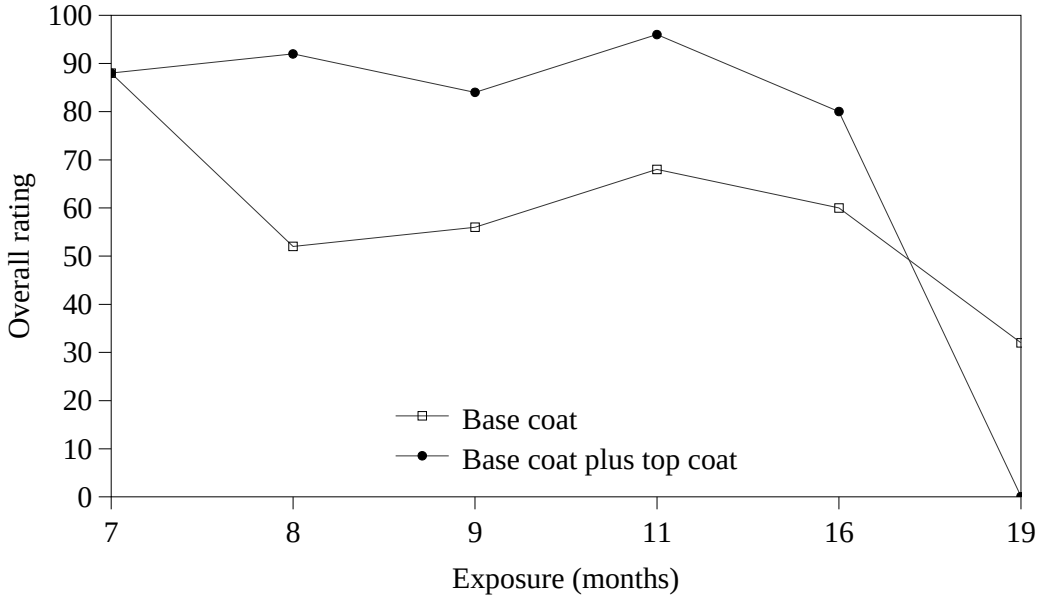


Figure 8.1 Overall fouling of base coat only and full system at Site 1, U.K.

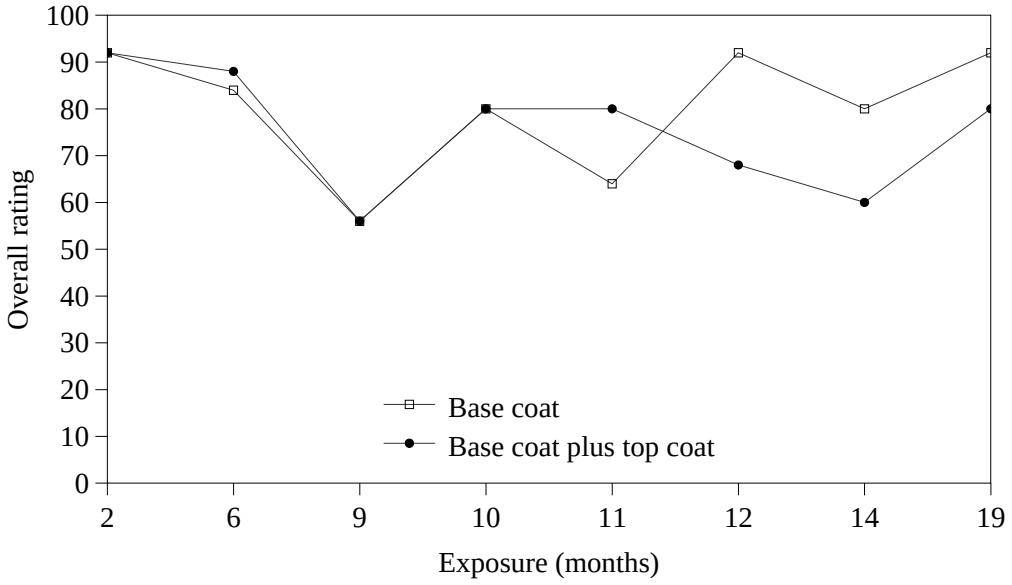


Figure 8.2 Overall fouling of base coat only and full system at Site 2, U.K.

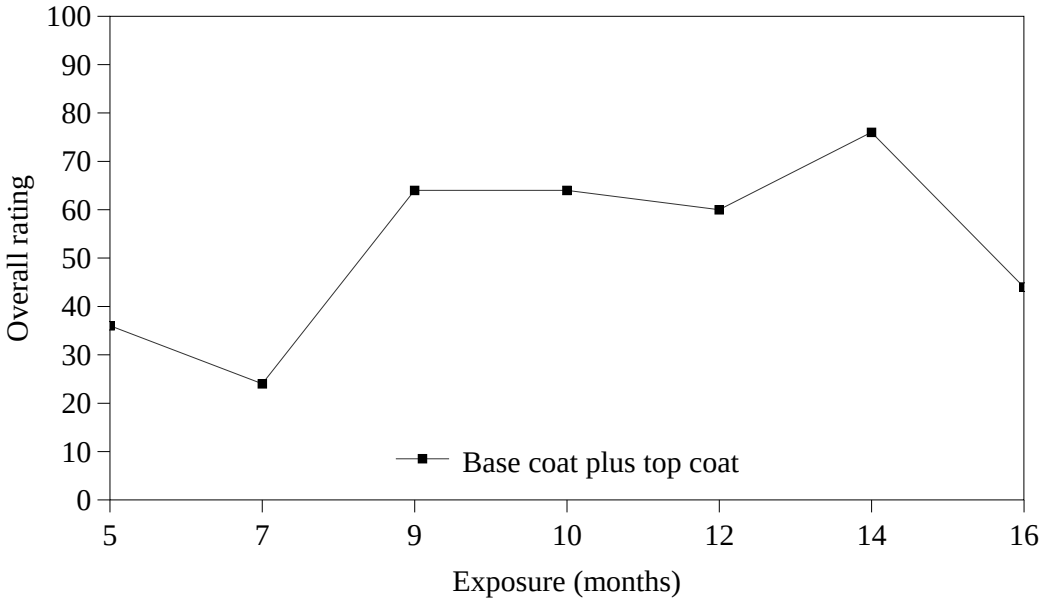


Figure 8.3 Overall fouling of full system only at Site 3, Singapore

The Author reported that at Site 1 (Figure 8.1), both coatings showed only light felt and slime fouling. The negative (blank) control strip was fouled with barnacles, weed, felt and hydroids, demonstrating a severe challenge. The test coatings showed a clear antifouling performance.

At Site 2 (Figure 8.2), the antifouling performance of both base coat and duplex system was reported as 'excellent', with only light slime being reported. The negative (blank) control strip was fouled with barnacles, weed, felt and hydroids, demonstrating a severe challenge.

At Site 3 (Figure 8.3) the fouling challenge was reported to be very severe. The negative (blank) control strip was fouled with a 'myriad of organisms' including gooseneck barnacles, barnacles, weed, felt, polyzoa and mussels. The duplex system showed slight fouling from slime, brown felt and trace barnacles.

The duplex system demonstrated clear antifouling performance based on an active ingredient combination of Cu_2O and zineb (Unpublished, 1996a).

A raft test was conducted by the same company according to the same standard test method (Appendix 4.3, Section 2). The results of the study were published. Only 1 test site in the UK was used for the trial. Zineb and Cu_2O were incorporated into a TBT-free ablative system in various ratios in order to assess efficacy. The active ingredient ratios in terms of percent volume are given in Table 8.5. It was not possible to determine active ingredient content in terms of % w/w.

Table 8.5 Active ingredient ratio of test formulations in terms of % volume

Paint	Cu_2O (% vol)	Zineb (% vol)
A	100	0
B	75	25
C	50	50
D	25	75
E	0	100

The paint formulations were applied to 1300 x 1000 mm 'Formica®' panels to a thickness of 100 µm. The panels were attached to wooden boards ready for immersion. Each board carried 25 panels, arranged in a randomised block at 5 immersion depths (5 panels at each depth). Two boards were used for each sequential month of immersion (10 replicates of each formulation were attached to each pair of wooden boards, making a total of 50 panels per sequential month). A total of 24 boards were used (sufficient for a 12 month study).

The trial was initiated in July 1986, and ran for 12 months. All boards were immersed on a raft at the same time. A set of two boards was then removed each month for inspection. Panels were inspected visually for the presence of weed and animal fouling. Slime was identified microscopically. Chlorophyll *a* determinations were carried out in order to estimate the amount of floral biomass that had grown on the panels. Ash weight determinations were also conducted, but the results were confounded by both the build-up of insoluble copper salts in the top layer of partly hydrolysed copolymer (the leached layer), and the removal of slime. Ash weight determinations were not therefore considered further. Biocide leaching measurements were also made after immersion for 1, 2, 3, 7, 10 and 12 months.

Paint E (active ingredient: 100 % zineb by volume) was rapidly colonised by *Achnanthes* spp., which formed a thick dark slime. Cyanobacteria were also present, as well

as macroalgae such as *Cladophora rupestris*, *Enteromorpha intestinalis* and *Ectocarpus* sp.. Later in the year barnacles and hydroids were also present.

Paint A (active ingredient: 100 % Cu_2O by volume) was mainly fouled by diatoms initially, replaced later by macroalgae such as *C. rupestris* and *E. intestinalis.*

Paints B, C and D were fouled early on in the trial with diatoms such as *Amphora coffeaeformis, Amphiprora hyalina, Navicula* spp. and *Stauroneis* sp.. There was no animal fouling, and macroalgal fouling was absent on paint D until month 11 (May).

The fouling levels on the panels as determined using chlorophyll *a* are given in Figure 8.4.

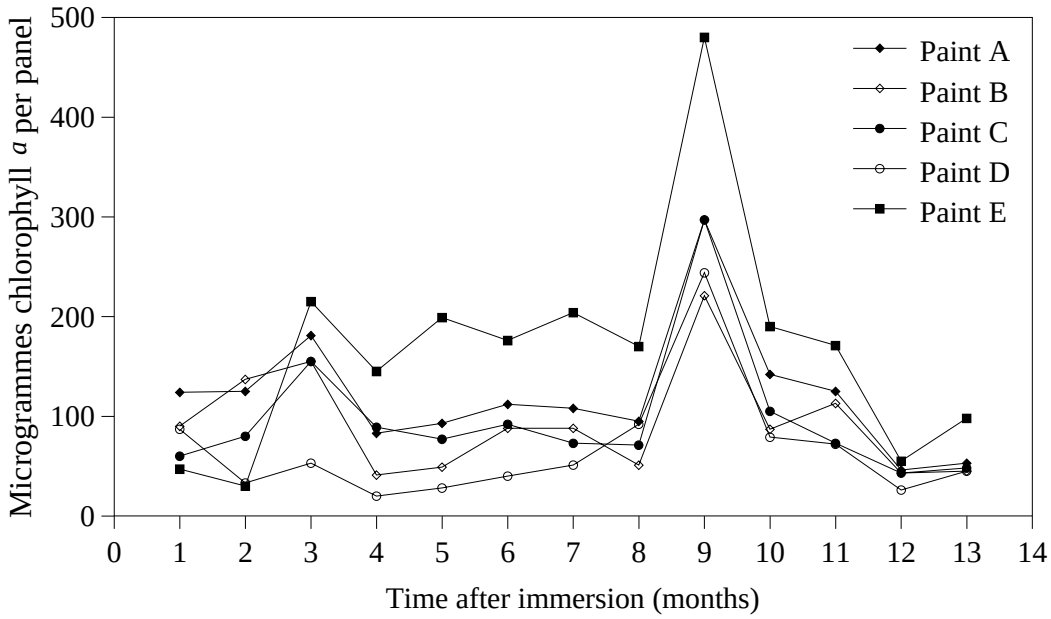


Figure 8.4 Fouling of test formulations in terms of amount of chlorophyll *a* per panel

There was a small peak in fouling 3 months after immersion (September) and a second larger peak 9 months into the study (March).

In overall terms, there was more fouling on paint E than on any others from 3 months until the end of the trial. Paint A was more fouled than the others at month 1. From month 5 to 7 only E was more fouled than A. The differences between paint A and paints B and C were reported by the Authors not to be statistically significant. There was little difference between paints B and C throughout the trial. Paint D was less fouled than all the others during months 3 to 7. B was the least fouled paint at months 8 and 9, but by month 10 D was less fouled than all of the others. This situation continued to the end of the trial.

Paint D leached less copper than the other copper-containing formulations during the first 8 months of the trial. For the first 12 months there was no difference in leaching rates of copper from A, B and C. However, after 12 months, leaching from B and C had reduced to the level of D, and the rate from A had increased to a level above the others.

Paint D leached more zineb/zineb derivatives than the others over the whole of the trial period. Two months after immersion E leached more zineb/zineb derivatives than B and C, but less than D.

The zineb-only formulation (paint E) was ineffective as an antifouling agent. The Cu_2O-only formulation (paint A) was more effective than E, but was not as good as formulations containing Cu_2O and zineb. Paint D was the most effective formulation during the first 7 months of the trial, and towards the end of the study period. The similar performance of D during the peak in fouling at month 9 suggests that the leaching rates of copper and zineb/zineb derivatives were below that necessary to control heavy Spring fouling.

In conclusion, zineb as the sole active ingredient in a TBT-free ablative antifouling product was ineffective, but when incorporated with Cu_2O it enhanced the performance of the formulation. The most effective combination was at a ratio of 75 % zineb:25 % Cu_2O by volume. The leaching results suggest that in the presence of zineb the release rate of copper can be reduced without loss of effectiveness (Hunter and Evans, 1991).

8.4.1.3 TBT ABLATIVE COATING TYPE

Two raft tests were conducted according to a company standard test method described in Appendix 4.3, Section 3. One test site in the UK and one in the USA were included in the trial. Panels of plastic (polyvinyl chloride [PVC]) measuring 200 x 400 mm were either coated with the test product or left uncoated (blank controls) and immersed for 18 and 17 months respectively. Duplicate panels were immersed at both test sites.

The first test examined the antifouling properties of a commercial antifouling product: Product A - a 19.95-20.43 % w/w TBTM/MMA copolymer (SPC) coating with 4.45 % w/w zineb and 4.0 % w/w Cu_2O.

The second test also examined the antifouling properties of a commercial antifouling product: Product B - a 19.81-20.35 % w/w TBTM/MMA copolymer (SPC) coating with 4.45 % w/w zineb and 4.0 % w/w Cu_2O.

The results of the studies are presented in Tables 8.6 and 8.7.

The data in Tables 8.6 and 8.7 show that the products containing TBTM/MMA copolymer plus Cu_2O and zineb performed well. In both tests, the blank panels were completely fouled by 3-4 months. It can be concluded that the zineb-containing products satisfactorily controlled fouling organisms on a static submerged surface (Unpublished, 1996b).

Table 8.6 Results of raft tests using product A

Antifouling product	UK		USA	
	Exposure (months)	Rating (%)	Exposure (months)	Rating (%)
Product A	10/03/91	-	11/12/90	-
	4	100	1	100
TBTM/MMA	6	97	3	100
19.95-20.43 %	9	97	5	100
Cu_2O 4.0 %	12	99	11	94
zineb 4.45 %	18	99	17	80
	10/03/91	-	11/12/90	-
	4	0	1	40-70
Blank panel	6	0	3	0
	9	0	5	0
	12	0	11	0
	18	0	17	0

The dates in the exposure columns indicate the start of the particular test

Table 8.7 Results of raft tests using product B

Antifouling product	UK		USA	
	Exposure (months)	Rating (%)	Exposure (months)	Rating (%)
Product B	10/03/91	-	11/12/90	-
	4	100	1	100
TBTM/MMA	6	100	3	100
19.81-20.35 %	9	99	5	100
Cu_2O 4.0 %	12	99	11	95
zineb 4.45 %	18	99	17	85
	10/03/91	-	11/12/90	-
	4	0	1	40-70
Blank panel	6	0	3	0
	9	0	5	0
	12	0	11	0
	18	0	17	0

The dates in the exposure columns indicate the start of the particular test

A raft test was conducted to examine the effect of removing zineb from a commercial formulation. The commercial formulation contained 25.00-26.16 % w/w TBTM/MMA copolymer plus 5.17-5.24 % w/w Cu_2O and 9.56-10.12 % w/w zineb. For the booster biocide-free formulation, the zineb was exchanged for calcium carbonate, an extender reported to have no biocidal activity. The exchange was made on a volume basis to keep the pigment volume concentration constant.

Steel panels were coated partly with the commercial product, and partly with the zineb-free formulation. The panels were immersed in the sea, and fouling was monitored on two occasions, reported to reflect typical in-service intervals for antifoulings on ships. The date of first immersion and the test site were not reported. No numerical assessments of fouling levels were reported. Instead, photographs of the test panels were provided.

The first inspection took place on the 21st of June 1995. It was apparent that there was very little if any fouling on the zineb-containing coating. The zineb-free coating was lightly fouled with slime (noted by Author).

The second inspection took place on the 11th of November 1995. The zineb-containing coating was lightly fouled, apparently with algae. The zineb-free coating was more heavily fouled, once again apparently with algae.

The poor documentation made evaluation and interpretation very difficult. Furthermore, it is apparent that the photograph taken in November 1995 does not show the exact same panels as seen in the picture taken in June 1995. These data do not provide satisfactory evidence of efficacy (Unpublished, 1996c).

A series of raft tests were conducted according to a standard test method, described in Appendix 4.3, Section 1. Three different TBT ablative formulations ('A', 'B', 'C') containing 10 - 50 % w/w Cu_2O, 6 - 31 % w/w TBT and 5 - 15 % w/w zineb were assessed. The panels were submerged to a depth of approximately 600 - 900 mm for a period of 80 weeks, commencing November 1990.

The results of the fouling of the formulations containing Cu_2O, TBT and zineb are presented in Figures 8.5, 8.6 and 8.7 and the blank (negative control) in Figure 8.8.

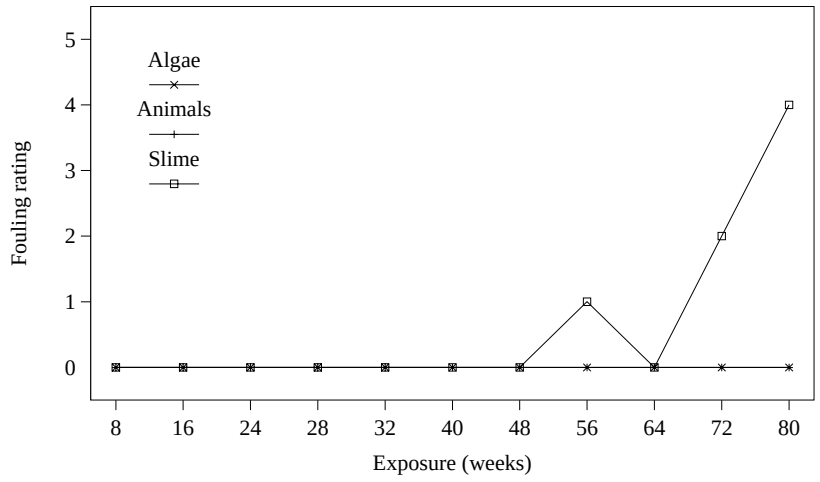


Figure 8.5 Raft testing results of an ablative antifouling formulation 'A' containing Cu_2O and zineb

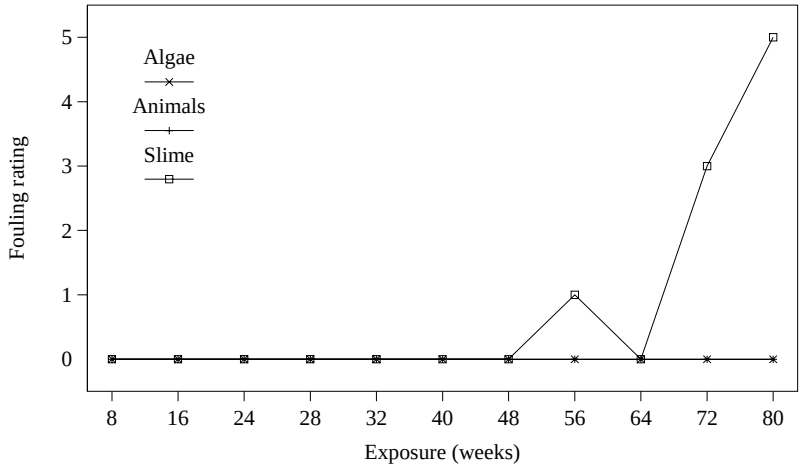


Figure 8.6 Raft test results of an ablative antifouling formulation 'B' containing Cu_2O, TBT and zineb

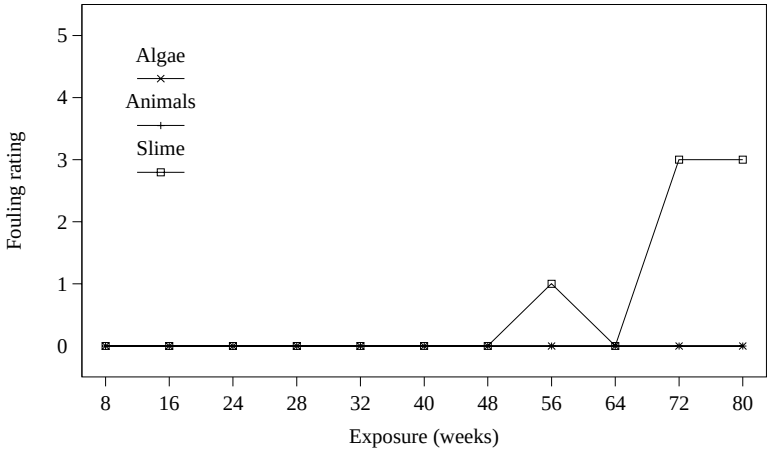


Figure 8.7 Raft test results of an ablative antifouling formulation 'C' containing Cu_2O, TBT and zineb

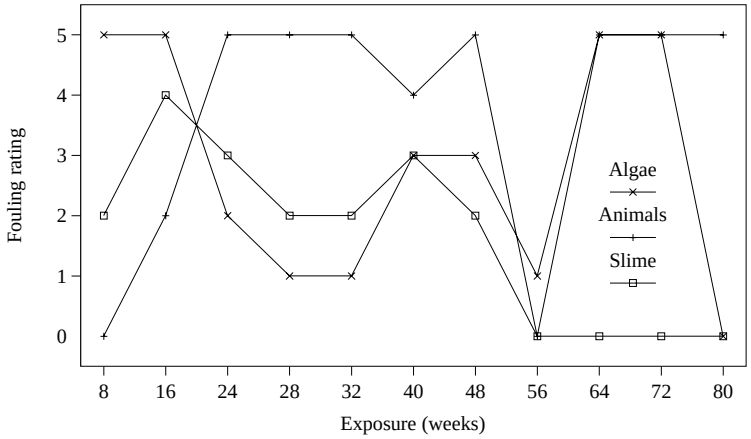


Figure 8.8 Raft test results of blank (negative) control panel

Figures 8.5 to 8.7 (Cu_2O, TBT and zineb formulations 'A', 'B' and 'C') show no fouling until after week 48, when slime fouling generally increased towards the end of the test to a maximum rating of 3 to 5. No animal or algal fouling was recorded during the test.

Figure 8.8 (blank panel) showed generally decreasing slime fouling over the period of the test. High initial algal fouling was replaced by high animal fouling until week 48. Following a brief reduction in total fouling at week 56, animal fouling increased to the maximum rating at week 64, and remained at this level until the end of the test period. Algal fouling also peaked at the maximum rating between weeks 64 and 72, after which it fell to 0. Fouling of the untreated panel was very dynamic over the test period, but generally remained at high levels.

The data suggest that under the conditions of this study, the test formulations prevented the establishment of algal and animal fouling species, and suppressed slime fouling for over half of the test period (Unpublished, 1993 b, c, d).

A raft test was conducted according to a standard test method, described in Appendix 4.3, Section 2. The results of the study were published. Only one test site in the UK was used for the trial. Zineb and Cu_2O were incorporated in 7 different proportions into a commercial TBT ablative system in order to assess efficacy. The formulation details in terms of % volume are given in Table 8.8. Please note that it was not possible to determine the content of each paint in terms of % w/w. The figures in Table 8.8 refer to ratios of active ingredients, and separately, to ratios of copolymer and plasticisers.

Table 8.8 Details of test formulations in terms of % volume

Paint	Vehicle composition (% by volume)			Cu_2O (% vol)	Zineb (% vol)
	Copolymer	Plasticiser A	Plasticiser B		
A	80	15	5	100	0
B	80	15	5	75	25
C	80	15	5	50	50
D	80	15	5	25	75
E	80	15	5	0	100
F	65	30	5	50	50
G	50	45	5	50	50

The paint formulations were applied by brush to 100 cm^2 abraded 'Formica®' panels to a thickness of 100 µm. For each paint formulation, 150 replicates were prepared, making a total of 1050 panels. Five replicates per formulation were attached to each of 30 wooden carrier plates ready for immersion. Each wooden plate carried a total of 35 panels, arranged randomly at 2 immersion depths (60 - 80 cm and 90 - 110 cm). Two boards were used for each sequential month of immersion, giving 10 replicates per month.

The trial was initiated in May 1986, and ran for 15 months. All boards were immersed on a raft at the same time. A set of two boards was then removed each month for inspection. Panels were inspected visually for the presence of weed and animal fouling. Slime was identified microscopically. Chlorophyll *a* determinations were carried out. Ash weight determinations were also conducted, but the results were confounded by both the build-up of insoluble copper salts in the top layer of partly hydrolysed copolymer (the leached layer), and

the removal of slime. Ash weight determinations were not therefore considered further. Biocide leaching measurements were also made after immersion for 1, 2, 3, 7, 10, 13 and 15 months. Statistical analyses of the data were provided.

Immersion level had no apparent influence on the species that fouled the test boards. There was no animal fouling on any of the paints during the trial.

Paint A (active ingredient:100 % Cu_2O by volume) was mainly fouled by *A. coffeaeformis*, *A. hyalina* and *Navicula* spp. throughout the trial. *Achnanthes* spp. and *Amphora veneta* were also present, but in fewer numbers. *Ulothrix flacca* was also present throughout the trial, and *C. rupestris* appeared after 3 months. Both of these latter species peaked in October. *E. intestinalis* was also present in low numbers, but increased towards the end of the trial.

Fouling on paints B, C and D consisted almost entirely of diatoms such as *A. coffeaeformis*, *A. hyalina* and *Navicula* spp.. There was a small amount of *U. flacca* plus *E. intestinalis* on B and C only.

Paint E (100 % zineb by volume) was colonised almost exclusively by *Achnanthes longpipes* 2 and 3 months after immersion (June and July), and *Achnanthes* spp. were the dominant fouling diatoms on this formulation over the first 10 months of the trial, forming an adherent slime that could not easily be removed. Seven months after immersion (November) macroalgae such as *E. intestinalis* increased markedly on this formulation. The extent of macroalgae fouling was much greater on E than it was on paints A to D.

No information on species present on formulations F and G were presented.

The fouling levels on the panels as determined using chlorophyll *a* are given in Figure 8.9 (Paints A to E) and Figure 8.10 (Paints C, F and G).

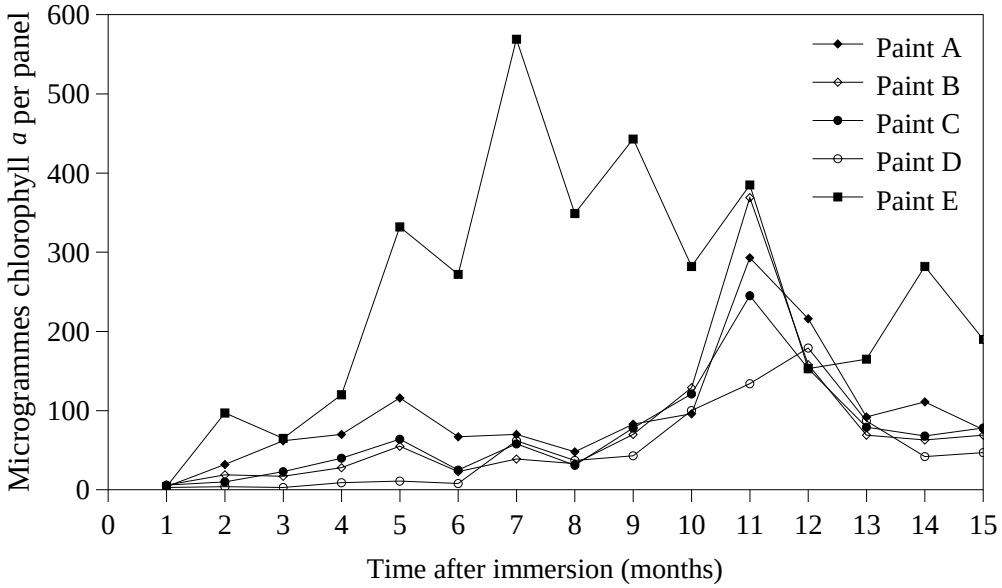


Figure 8.9. Fouling of test formulations A to E in terms of amount of chlorophyll *a* per panel

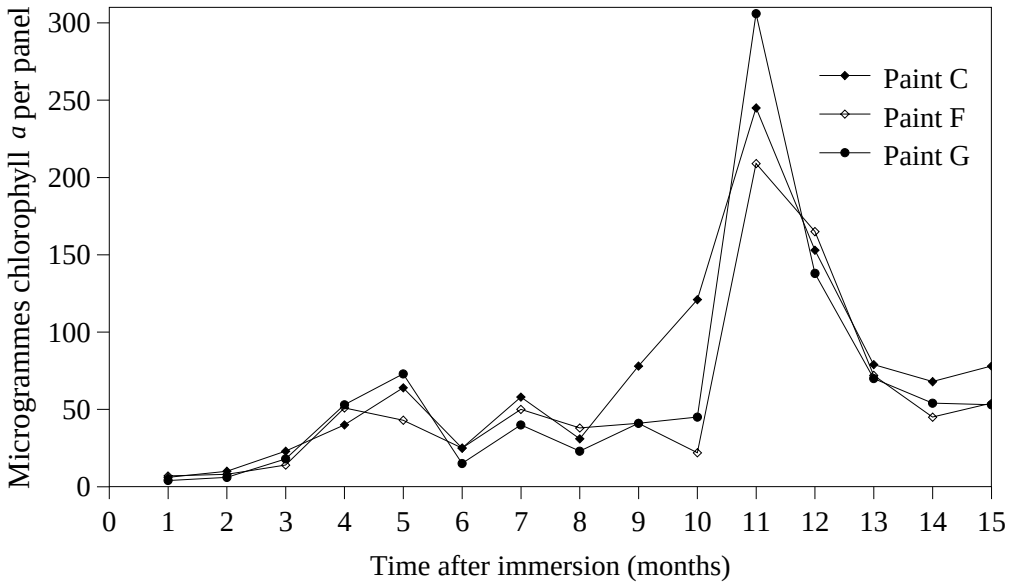


Figure 8.10. Fouling of test formulations C, F and G in terms of amount of chlorophyll *a* per panel

Figure 8.9 shows that there was a moderate peak in fouling 5 months after immersion (September) and a second larger peak 11 months into the study (March). Paint E also showed a fouling peak 7 months after immersion (November).

Figures 8.9 and 8.10 show that in overall terms, there was more fouling on paint E than on B to D from 3 months (July) until month 10. E was also generally more fouled than paint A during the first 10 months of the trial. Paint A was more fouled than B to D from month 2 to 8. Paint D was less fouled than A, B, C and E during months 3 to 6. There was no statistically significant difference in fouling between paints B and C during the trial period. Similarly, there was little difference in the antifouling performance of C, F and G throughout the study period.

After 5 months (September) and 9 months (January) immersion, panels at 60 to 80 cm were statistically significantly more fouled that panels at 90 to 110 cm.

With regard to leaching, 1 to 3 months after immersion paint A was leaching statistically significantly more TBTO than B to E, and D and E were leaching statistically significantly less TBTO than A, B and C. After 7 to 10 months there was no difference between the TBTO leaching rates of A to E, after 15 months A and D were leaching less TBTO than E and C. In the case of the paints with a different vehicle composition but the same pigment ratios (C, F and G), after 1 to 2 months immersion TBTO leaching was greatest from G and least for C. However, after 3 months there was no difference in leaching rates between these formulations.

One and 2 months after immersion A and B were leaching statistically significantly more copper than C and D. This continued except during months 10 and 13. F and G leached more copper than C, A and B.

Paint E leached more zineb than B and C until 13 months after immersion. D leached more zineb than B and C until after month 7. E leached slightly more zineb than D throughout the trial. There was no statistically significant difference between zineb leaching rates of C, F and G.

Paint D was the most effective formulation over the first 6 months of the trial. For the first half of the trial, this paint leached less copper and TBTO than A and B, but more zineb than B and C. After 7 months, the zineb release rate of D had dropped, and by 13 months it was the same as the other zineb-containing formulations. The TBTO release rate also dropped after 7 months, whilst copper release did not drop after month 2. The drop in release rates of TBTO and zineb accounts for the development of the slime layer 7 months after immersion.

Paint E (only zineb in addition to copolymer) was the least effective over the first 8 months of the study period. The high zineb release rates shown by this formulation were unable to prevent fouling. The low TBTO release rate during the first 3 months may have also contributed to rapid colonisation.

Copper plus copolymer (Paint A) was more effective that E, but less so than the zineb/copper formulations after 9 months. The high release rate of TBTO from this formulation suggests that in the others, the presence of zineb was reducing the rate of polymer hydrolysis.

Increased plasticiser levels in paints F and G had little effect on the systems' performance.

As an overall conclusion, zineb as the sole additional active ingredient in an TBT copolymer antifouling product was relatively ineffective, but it did enhance the performance of the Cu_2O containing system. The most effective combination was at a ratio of 75 % zineb:25 % Cu_2O by volume in the coploymer system. It was noted in the paper that zineb degrades to form products that are in themselves toxic, and it may be the nature and behaviour of the derivatives that determine efficacy against target organisms. It was also noted that degradation of zineb may occur during its passage through the leached layer of paint, and that an understanding of this process is needed (Hunter and Evans, 1991).

8.5 OVERALL SUMMARY OF EFFICACY DATA

No laboratory screening data were submitted.

The majority of the relevant Approval Holders provided efficacy test data generated using raft tests. The studies were conducted on a range of formulations. In the case of the TBT ablative formulations, it was possible to determine that these were representative of current approvals with respect to active substance levels of the organic 'booster biocide' zineb. The biocide was present in combination with copper (Cu_2O) as the principal biocide.

Studies were provided in support of antifouling products formulated using TBT and TBT-free ablative technologies. No data were provided specifically in support of products based on the contact leaching systems.

The raft studies were performed over periods of one or more 'seasons' at a number of test sites at widely differing geographical locations. The fouling challenges were equally diverse.

It can be concluded, from the various raft test studies, that the products based on TBT and TBT-free ablative coatings that contained zineb as a 'booster biocide' plus copper as either the sole principal biocide or in conjunction with other principal biocides achieved a satisfactory level of antifouling activity against both algae and animals. This performance was against a diverse fouling challenge that, depending on test site and environment, could often be severe. From the data provided, however, it was not possible to determine if the levels of zineb used in the TBT-free ablative formulations were representative of current approvals.

No field data (such as vessel patch or panel tests) were submitted by any of the Approval Holders in support of their products.

It should be noted that, particularly for ablative technologies, a static raft test provides a harsh challenge since effects due to physical ablation and/or polishing are minimised. For these types of coatings it can be expected that antifouling performance will be further improved on a moving vessel due to the ablative/polishing effects.

8.6 DATA REQUIREMENTS

To support the continued use of TBT-free ablative formulations, Approval Holders should submit confirmation that data provided are representative of current approved products. This evidence should be in the form of full details of formulations used in the raft trials that have been reported. If these data are not provided, then further raft test or alternatively field trial/in service monitoring data are required. As appropriate these data should cover:

(i) The efficacy of TBT-free ablative antifouling formulations that contain zineb.

To support the continued use of contact leaching antifouling products containing zineb, further raft test or alternatively field trial/in service monitoring data are required. These data should cover:

(ii) The efficacy of contact leaching antifouling formulations that contain zineb.

In all cases, Approval Holders should provide data in support of the coating type(s) that best befits their products. If a product is assigned to a different and more accurate coating type by an Approval Holder, and is, as a consequence, not supported by existing information, appropriate efficacy data must be submitted.

9 RECOMMENDATIONS AND DATA REQUIREMENTS

A partial review of zineb was considered in October 1999 and data requirements addressing analytical and physicochemical endpoints and the skin penetration of zineb in antifouling products were identified. Personal protective equipment was specified as a condition of approval. Full details of these requirements and conditions are set out in Appendix 1.

A full review of zineb (which considered all available data on physico-chemical, toxicological, environmental fate and behaviour, ecotoxicology and efficacy) was considered by the Committees in September 2000 and Ministers agreed to the following recommendations:

> Provisional approval should be allowed to continue for both amateur and professional use of antifouling products containing a maximum formulation concentration of 20 % w/w zineb.

Certain restrictions have been placed on the above recommendation and additional data requirements were also set:

1. Approval Conditions on amateur use of zineb

a. Amateur use by brush and roller may continue provided that a suitable precautionary phrase stating 'WEAR SUITABLE PROTECTIVE GLOVES when applying' appears on the product label.

b. Due to concerns over the skin sensitisation potential of zineb, amateur use by airless / conventional spray is considered unacceptable so this use must be removed from product labels and associated literature.

2. Approval conditions on professional use of zineb

a. Professional use by brush, roller and conventional / airless spray may continue.

b. In order to reduce exposure for professional operator and others, the approval conditions are to be amended to include the following requirements:

> All professional operators exposed to antifouling products containing zineb should wear a disposable coverall with hood (providing head protection) and a second overall beneath this coverall of a contrasting colour to the antifouling product being applied. All bare skin should be covered. The disposable coverall should normally be used for no more than one spraying session. The second overall should be changed regularly and whenever paint breakthrough has been detected.
>
> Professional operators working with antifouling products containing zineb should wear impermeable gloves of a type recommended by the paint manufacturer as suitable for use with that formulation. These gloves should be changed regularly e.g.

after 1-2 day's use. Operators should wear impermeable (and non-slip) footwear that protects the lower leg.

Professional operators (sprayers) exposed to antifouling products containing zineb must wear respiratory protective equipment (RPE). Appropriate RPE includes air-fed respiratory protective equipment with combined protective helmet and visor to protect the skin of the head and neck. Impairment of vision should be avoided. For other workers, the need for RPE should be informed by COSHH assessment. Unprotected persons should be kept out of treatment areas.

c. These approval conditions will appear on schedules for all approved products containing zineb and the following precautionary phrases must appear on product labels:

WEAR SUITABLE PROTECTIVE CLOTHING (COVERALLS OF A CONTRASTING COLOUR TO THE PRODUCT BEING APPLIED, UNDERNEATH A DISPOSABLE COVERALL WITH HOOD), SUITABLE GLOVES AND IMPERVIOUS FOOTWEAR THAT PROTECTS THE LOWER LEG.

DISPOSE OF PROTECTIVE GLOVES after use.

DO NOT BREATHE SPRAY MIST.

WEAR SUITABLE RESPIRATORY EQUIPMENT (such as air-fed respiratory equipment with combined protective helmet and visor) when spraying.

UNPROTECTED PERSONS SHOULD BE KEPT OUT OF TREATMENT AREAS.

3. Data Requirements for continuing use of zineb

a. Two-year storage stability studies at ambient temperatures on representative formulations to be submitted (if these studies are unavailable at this time, then accelerated studies should be submitted and 2-year studies to support full approval of the compound).

b. Approval Holders should provide a short-term toxicity study on zineb so that a NOAEL more appropriate to the pattern of amateur use may be identified. This should be a 28-day oral study in the rat with periodic interventions including full histopathological examinations, particularly of the target organs.

c. Leaching rate of zineb from a treated surface using representative formulations to be submitted.

d. Confirmation of aquatic metabolism of zineb in marine sediment-water systems, including full characterisation of the metabolites (addressing effects of copper ions) to be submitted.

e. Chronic exposure of the parent compound to a suitable marine species to be submitted after meeting data requirements c. and d. Significant reductions in the leaching rate and/or degradation rate may reduce the predicted environmental concentrations, negating the need for this study to be carried out to reduce the current safety factor.

f. Once the fate of zineb (in addition tot he significance and persistence of its metabolites) has been established, additional ecotoxicology plus fate and behaviour data may be required.

g. Raft test or alternatively field trial/in-service monitoring efficacy data to be submitted.

Ministers agreed to the above recommendations.

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APPENDIX 1

DATA REQUIREMENTS AND CONDITIONS OF APPROVAL SET IN 1999

Ministers agreed that the provisional approval for the professional and amateur use of antifouling products containing up to 20 % w/w zineb should be permitted to continue, subject to the following conditions and data requirements:

a. That all professional operators (for all application methods) wear a disposable coverall with hood (providing head protection) and, beneath this, a second overall of a contrasting colour to the antifouling product being applied. All bare skin should be covered. The disposable coverall should be used for no more than one spraying (or other means of application) session. The overall should be changed regularly and whenever product break-through has been detected. In addition, gloves should be changed after each application operation.

b. That professional sprayers should also wear RPE. Appropriate RPE includes air-fed respiratory equipment with combined protective helmet and visor. For non-spraying operatives (pot-men and other workers), the need for RPE should be informed by a COSHH assessment. Unprotected people should be kept out of treatment areas. Labels should include a warning not to breathe spray mist.

c. That Approval holders should be required to provide the following data within one year (except where specified):

Physical Chemistry

Supplier 1:

1. Results from a typical batch analysis conducted on the technical material, with the associated test report (e.g. chromatographic traces should be included).

2. The vapour pressure at 25 ^{o}C for technical zineb, with full test reports submitted (if this cannot be measured a reasoned case should be provided with the test report for the estimated value).

3. Full test reports for the melting point (including confirmation that the decomposition temperature is 157 ^{o}C) and solubility in water.

4. Details of an analytical method to determine the zinc in technical zineb. Typical examples of the data generated from using this technique should be provided.

Supplier 2:

1. Spectral evidence for the structure of zineb (e.g. UV, IR) conducted on the technical material.

2. Results from a typical batch analysis conducted on the technical material, with the associated test report (e.g. chromatographic traces should be included).

3. The following physico-chemical endpoints for technical zineb, with full test reports: Melting point, relative density at 20 ^{o}C, surface tension of the active ingredient in aqueous solution at 25 ^{o}C, vapour pressure at 25 ^{o}C, solubility in water at 25 ^{o}C, partition co-efficient (log P_{ow}) at 25 ^{o}C (if this cannot be measured a reasoned case should be provided) and flammability.

4. Protocol reports, plus typical experimental results, for the analytical method submitted for the determination of zineb in the technical material.

5. Details of an analytical method used for the determination of the additive (hexamethylenetetramine) in the technical material and of analytical methods used for the determination of impurities in the technical material.

6. Details of an analytical method for the determination of zineb in water (limit of quantitation should be 0.1 mg l^{-1}).

Typical examples of the data generated from using the above analytical methods should also be submitted.

<u>All approval holders:</u>

Two-year storage stability studies on representative formulations (if these studies are not already available, accelerated studies should be submitted and two-year studies provided to support full approval).

<u>Approval holder W:</u>

Details of an analytical method for the determination of the active ingredient in antifouling products.

<u>Approval holders X, Y and Z:</u>

Examples of the data generated from using the analytical methods submitted for the determination of the active ingredient in antifouling products, including examples of the data generated from using the method.

<u>Mammalian toxicology/exposure data</u>

All Approval Holders for professional antifouling products containing zineb should submit a study of the skin penetration of zineb from formulations representative of approved antifouling products. This study may be *in vivo* or *in vitro*.

These requirements have been conveyed to approval holders but the deadline for data submission had not yet expired when the ACP considered the full review of zineb in 2000.

APPENDIX 2

CALCULATIONS FOR EXPOSURE ASSESSMENT

The following database models were initially presented in guidance document EH74/3 (HSE 2000).

Defaults assumed - Spray Applications:

- Operator weight 60 kg
- Operator respired volume 1.25 m^3 h^{-1}
- Median shift length 3 h

Table 2.1 Summary of exposure data for sprayers (mg in-use product h^{-1})

	Frequency (%)	Central	95th % or * worst case
Potential dermal	100	6170	**44700**
Weighted indicative value		**6170**	
Clothing penetration	93	4.5 %	
Weighted indicative value		**4 %**	
In-glove exposure	100	60	**241**
Weighted indicative value		**60**	
Inhalation exposure	91	6.6 mg m^{-3}	**64.6 mg m^{-3}**
Weighted indicative value		**6 mg m^{-3}**	

Table 2.2 Summary of exposure data for pot-men (mg in-use product h^{-1})

	Frequency (%)	Central tendency	95th % or * worst case
Potential dermal	100	2940	**15000**
Weighted indicative value		**2940**	
Clothing penetration	59	7 %	
Weighted indicative value		**4 %**	
In-glove exposure	100	34.9	**1380 ***
Weighted indicative value		**35**	
Inhalation exposure	68	0.9 mg m^{-3}	**42 mg m^{-3} ***
Weighted indicative value		**0.6 mg m^{-3}**	

Table 2.3 Summary of exposure data for ancillary workers (mg in-use product h^{-1})

	Frequency (%)	Central tendency	95th % or * worst case
Potential dermal	100	885	**3470**
Weighted indicative value		**885**	
Clothing penetration	59	7 %	
Weighted indicative value		**4 %**	
In-glove exposure	100	35	**180 ***
Weighted indicative value		**35**	
Inhalation exposure	50	1.7 mg m^{-3}	**4.8 mg m^{-3} ***
Weighted indicative value		**0.8 mg m^{-3}**	

The original reports from which data in these tables are derived are available for scrutiny at HSE Bootle. The number of exposure data and the exposure ranges are shown in Table 2.4.

Table 2.4 Summary of exposure data and ranges - antifoulant spraying

Exposure	Sprayer		Pot-men and other operators	
	No. of data	**Range**	**No. of data**	**Range**
Potential dermal exposure (mg product h^{-1})	29	52.2 - 74100	28	16.2 - 18200
In-glove exposure (mg product h^{-1})	19	0.18 - 252	17	0.31 - 1380
Inhaled (mg product m^{-3})	20	0.04 - 79.4	16	0.04 - 41.6

Table 2.5 Exposure estimate (product) - sprayers

	Central tendency	**Worst case**
Coveralls		
Potential dermal exposure, mg h^{-1}	6170	44700
Work time per day, h	3	3
Daily deposit on clothes, mg	18510	134000
Penetration, %	4	4
Dermal exposure to product, mg	740	5360
Gloves		
Dermal exposure inside, mg h^{-1}	60	241
Work time per day, h	3	3
Dermal exposure to product, mg	180	723
Total dermal exposure		
Antifouling product, mg	**920**	**6080**
Inhaled		
Concentration, mg m^{-3}	6	64.6
Work time per day, h	3	3
Inhaled air volume, m^3	3.75	3.75
Inhaled product, mg - no RPE	**22.5**	**242**
Inhaled product, mg - RPE x PF 50	**0.45**	**4.84**

PF - RPE protection factor

Table 2.6 Exposure estimate (product) - pot-men

	Central tendency	Worst case
Coveralls		
Potential dermal exposure, mg h^{-1}	2940	15000
Work time per day, h	3	3
Daily deposit on clothes, mg	8820	45000
Penetration, %	4	4
Dermal exposure to product, mg	353	1800
Gloves		
Dermal exposure inside, mg h^{-1}	35	1380
Work time per day, h	3	3
Dermal exposure to product, mg	105	4140
Total dermal exposure		
Antifouling product, mg	**458**	**5940**
Inhaled		
Concentration, mg m^{-3}	0.6	42*
Work time per day, h	3	3
Inhaled air volume, m^3	3.75	3.75
Inhaled product, mg - no RPE	**2.25**	**158***

* These values reduce to 3.84 mg m^{-3} and 14.4 mg respectively when the top data point is disregarded as proposed in the text

Table 2.7 Exposure estimate (product) - ancillary workers

	Central tendency	Worst case
Coveralls		
Potential dermal exposure, mg h^{-1}	885	3470
Work time per day, h	3	3
Daily deposit on clothes, mg	2660	10400
Penetration, %	4	4
Dermal exposure to product, mg	106	416
Gloves		
Dermal exposure inside, mg h^{-1}	35	180
Work time per day, h	3	3
Dermal exposure to product, mg	105	540
Total dermal exposure		
Antifouling product, mg	**211**	**956**
Inhaled		
Concentration, mg m^{-3}	0.8	4.8
Work time per day, h	3	3
Inhaled air volume, m^3	3.75	3.75
Inhaled product, mg - no RPE	**3**	**18**

Defaults assumed - Brush and roller applications

- User weight 60 kg
- Operator respired volume 1.25 m^3 h^{-1}
- Median job duration 1.5 h
- Professional chandlers undertake the same tasks as amateur users of antifoulants

Table 2.8 Summary of exposure data for amateurs (mg h^{-1} in-use product)

	Frequency (%)	Central tendency	95th % or * worst case
Potential dermal	100	1020	**6480 ***
Weighted indicative value		**1020**	
Clothing penetration	11	42 %	
Weighted indicative value		**5 % ****	
In-glove exposure	100	31.2	**1110 ***
Weighted indicative value		**31**	
Bare hand exposure	100		**4400 ***
Inhalation exposure	44	0.04 mg m^{-3}	**0.11 mg m^{-3} ***
Weighted indicative value		**0.02 mg m^{-3}**	

** It is possible that amateurs wear only minimal clothing when applying antifouling products. A weighted indicative value for penetration in such cases is a default 50 %.

Table 2.9 Exposure estimate (product) - amateur users

	Central tendency	Worst case
Coveralls		
Potential dermal exposure, mg h^{-1}	1020	6480
Work time per day, h	1.5	1.5
Daily deposit on clothes, mg	1530	9720
Penetration, %	5	50 **
Dermal exposure to product, mg	76.5	4860
Gloves		
Dermal exposure inside, mg h^{-1}	31	4400 **
Work time per day, h	1.5	1.5
Dermal exposure to product, mg	46.5	6660
Total dermal exposure		
Antifouling product, mg	**123**	**11500**
Inhaled		
Concentration, mg m^{-3}	0.02	0.11
Work time per day, h	1.5	1.5
Inhaled air volume, m^3	1.88	1.88
Inhaled product, mg - no RPE	**0.04**	**0.21**

** Worst case - no gloves, minimal clothing.

Table 2.10 Exposure estimate (product) - professional chandler

	Central tendency	Worst case
Coveralls		
Potential dermal exposure, mg h^{-1}	1020	6480
Work time per day, h	1.5	1.5
Daily deposit on clothes, mg	1530	9720
Penetration, %	5	5
Dermal exposure to product, mg	76.5	486
Gloves		
Dermal exposure inside, mg h^{-1}	31	1110
Work time per day, h	1.5	1.5
Dermal exposure to product, mg	46.5	1670
Total dermal exposure		
Antifouling product, mg	123	2160
Inhaled		
Concentration, mg m^{-3}	0.02	0.11
Work time per day, h	1.5	1.5
Inhaled air volume, m^3	1.88	1.88
Inhaled product, mg - no RPE	0.04	0.21

APPENDIX 3

USE OF SAFETY FACTORS IN ENVIRONMENTAL RISK ASSESSMENT

By assuming that ecosystem sensitivity depends on the most sensitive species and that by protecting the community structure the function is also protected, single-species acute toxicity data are extrapolated to ecosystem effects. The most sensitive species is generally established in the laboratory.

Safety factors are applied to the generated toxicity data to predict a concentration below which the probability of environmental effects is considered sufficiently low to accept the proposed use of a product. It should be noted that this is not a concentration below which an a.i. is considered to be safe. In deciding the size of the safety factor to use with data several uncertainties must be addressed:

Inter-species variations,

Acute to chronic toxicity extrapolation,

Laboratory to field data extrapolation.

The following safety factors could have been, and were, used during the environmental risk assessment of the booster biocides:

- 1000* applied to the lowest L/EC_{50} of the core data set, i.e. acute toxicity of the a.i. to fish, daphnids and algae. A safety factor of 1000 is conservative and protective, and is used when only the core set of acute data is available.

- 50* applied to the lower NOEC from long-term toxicity data for two species from two taxonomic groups.

- 10* applied to the lowest NOEC from long-term toxicity data for fish, daphnids and algae. With increasing availability of acute and chronic data the safety factor of 50 or even 10 can be considered.

- 100** applied to the lowest NOEC from long-term toxicity data for two or more species from two or more taxonomic groups when the majority of the assessed data are published rather than company supplied safety data.

* These safety factors are as described in the EURATGD document.

** This safety factor has been previously used by HSE, because the quality of published data is considered to be less assured than that of safety data generated in a quality-controlled laboratory. It is a precautionary approach that has been previously accepted by the IDS and ACP.

(EURATGD, 1996)

APPENDIX 4.1

CURRENT ANTIFOULING COATING TYPES

Coating type	Description and Properties
Soluble matrix (conventional)	In coatings of this type, the biocide(s) have been physically mixed ('freely associated') into a rosin matrix. Upon exposure to sea water, the slightly acidic matrix slowly dissolves releasing the biocide(s) into the water (sea water is slightly alkaline (pH 8) and the acidic matrix readily dissolves). Continuous dissolution of the coating surface occurs, resulting in fresh biocide(s) being released until eventually the film is exhausted. The soluble matrix coatings have poor mechanical properties which limit film thickness and hence the coating lifetime attainable to approximately 12-18 months. As the matrix rosin is a natural product, batches differ and therefore coating lifetime is unpredictable.
Insoluble matrix (contact leaching or long life)	Within this type of coating, the binder or matrix is insoluble, and the biocide(s) is physically mixed into the matrix (often at higher concentrations than is the case with the conventional coatings). As sea water enters the paint film the biocides are released by dissolution and diffusion from within the insoluble matrix. This type of coating has a high initial release rate, which decreases exponentially with time as the biocide(s) has further to travel through the paint film. This release process continues until exhaustion of the coating. The higher mechanical strength obtained with these coatings allow applications of thicker systems, and as a consequence, coating lifetimes of approximately 24 months are attainable.
Ablative (self polishing copolymer) TBT coatings	In this type of coating the TBT biocide is chemically bound to the binder of the paint, a methacrylic acid/methylmethacrylate copolymer matrix into which other biocides can be incorporated. The copolymer hydrolyses at a predictable rate in sea water (depending on temperature, pH and rate of movement of a vessel through water), releasing the biocide(s) into the surrounding water, and creating a localised concentration at the paint surface discouraging the growth of settling organisms. This hydrolysis results in a softening of the surface layer of the copolymer and, together with the physical wearing away of the binder by the action of passing sea water (‘polishing’), exposes fresh surface layers. This mode of action with biocide release and polishing rate are both dependent on the same (chemical) process. The paint film thus smoothes, reducing drag and turbulence until eventually, through these processes, the whole of the coating is exhausted. After initial rapid release, a steady biocide release is achieved; the life of the coating is proportional to its thickness and is accurately predictable. The copolymer has a high mechanical strength, allowing build up of very thick systems and hence correspondingly long coating lifetimes, typically up to 5 years.

Coating type (continued)	Description and Properties (continued)
Ablative (polishing copolymer) Tin-free coatings	Coatings of this type rely on soluble medium, such as rosin, in combination with insoluble polymers to form a matrix that wears away *physically* at a controlled rate. The biocide(s) is mixed into the matrix and released by dissolution at a rate determined by the rate of physical ablation of the polymer. The physical ablation process is less controlled and predictable than the chemical ablation process. Therefore the steady release rate, predictable life, smoothing and recoating properties of the TBT copolymer coatings are difficult to achieve with this group of coatings. These tin-free copolymer coatings have, to date, demonstrated that the better performers within this group of products can achieve dry docking intervals of 3 years.

APPENDIX 4.2

ANTIFOULING COATINGS: METHOD FOR THE GENERATION OF EFFICACY DATA

Scope

The purpose of this test method is to determine, by raft testing, the effectiveness of an antifouling coating relative to an untreated substrate.

In static raft testing, the fouling challenge varies between raft sites, between positions on rafts, and from season to season etc. The results obtained by the raft testing described in this method are purely an indication of a product's ability to prevent the settlement of fouling organisms under static conditions at a particular fouling challenge relative to an uncoated substrate tested under exactly the same conditions.

The present method is not applicable to evaluate complete coating systems or the relative lifetime of coatings. Thus, the results obtained by the described method are not serving to demonstrate actual performance in service. The results obtained can therefore not be adopted for the relative assessment of products.

Principle of Method

In this method the antifouling paint is applied onto one or more raft test panels and exposed from the raft along with an uncoated substrate. Raft panels are made out of hard material; typically plastic or another inert material. Brush, roller, spray or other specialised equipment like a bar-type applicator may be used to apply the paint. The minimum dry film thickness should be in the range 90 -100 µm although thicker films may be applied depending on the decided use pattern of the antifouling coating. Minimum area for immersion is 150 - 200 cm^2. After proper drying, the panels in test are mounted on the raft and immersed in the marine environment. Depending on the intended use pattern of the antifouling paint, one or more raft sites may be used for exposure.

At given time intervals, the panels are assessed for presence of fouling organisms. Assessment of slime, algae, and animal fouling is quantitative, or, as a minimum, semi-quantitative. The duration of the test may vary. To demonstrate efficacy, the minimum immersion time for testing is 6 months. Depending on the intended use pattern of the paint under test, the immersion time may be extended.

Resistance to fouling at the raft site is demonstrated if there is no colonisation of the surface, or colonisation is minimal relative to the uncoated substrate.

Fouling assessment data are reported in the form of a raft performance report (Unpublished, 1993a).

APPENDIX 4.3

SECTION 1: RAFT TEST METHOD 1

The test product is applied by hand to a 200 cm^2 acrylic panel. Panels are coated on one side with one coat giving a dry film thickness of approximately 80 - 100 µm (the actual area of panel coated is approximately 132 cm^2). A second, identical panel is left blank as a negative control.

After the panels are painted they are left to dry for 1 week before sealing in a plastic bag for transport to the test facility on the Mediterranean for immediate immersion. The panels are hung vertically on a test raft floating on the surface of the sea, and submerged a minimum of 1.5 cm apart, facing in the same direction. The site is reported to represent medium to heavy fouling conditions. No further environmental details of the test site have been provided.

Evaluation of panel fouling is usually undertaken monthly, and photographs (submitted to HSE) are taken every second month. The panels are removed from the sea and sprayed with a hand held shower at a distance of 60 cm to remove silt and loosely attached organisms. A visual assessment is made for slime (composed of diatoms and initial algal germination), algal and animal fouling. The percentage of fouling for each of the above 3 groups of organisms is rated as indicated in Table 4.1.

Table 4.1 Rating of fouling assessment in raft tests conducted by the company

Percentage of Fouling	Rating
No fouling	0
0 - 2	1
2 - 5	2
5 - 25	3
25 - 50	4
50 - 100	5

The sum of all the fouling rates for each group gives an estimation of the antifouling properties of the test product.

The performance of the products is contrasted against a negative (blank) control.

SECTION 2: RAFT TEST METHOD 2

Two coats of the product are applied by brush to a marine plywood panel. Two separate panels are coated for immersion at each test site. A negative (blank) control panel serves as a control at each test site.

UK test site 1

Typical water temperatures, at UK test site 1, range from a winter low of about 9 ^{o}C in February/March to a summer high of about 19 ^{o}C in August. Salinity is generally in the range 32-34 % but may on occasion drop below 30 % immediately after a heavy rainfall. The sea water pH is generally in the range 8.10-8.25. The tidal range is 2 m at low tide to 7 m at high tide.

The fouling community is reported to be diverse, with the greatest challenge in July/August. Because of the mild climate, the fouling season extends from April to October. Particularly abundant fouling organisms include slimes (Acananthes and Amphora spp.), algae/weeds (*Enteromorpha* and *Ectocarpus* spp.), barnacles (*Eliminus modestus* and *Semibalanus balanoides*), molluscs (*Mytilus edulis*) and hydroids (*Tubularia* spp.). Additional organisms include polyzoans, tubeworms (Serpulids), sponges (*Halichondria* spp.) and tunicates (*Botyllus* spp.).

UK test site 2

This is located in temperate waters on an estuary. Typical water temperatures, at UK test site 2, range from a winter low of about 8 ^{o}C in February/March to a summer high of about 20 ^{o}C in August. Salinity is generally in the range of 32-34 % but may on occasion drop below 30 % immediately after heavy rainfall. The sea water pH is generally in the range 8.10 - 8.25.

A diverse range of fouling challenge is present at the site, particularly abundant fouling include slimes (*Acananthes* and *Amphora* species), barnacles (*E. modestus*), molluscs (*M. edulis*), amphoids (Jassa) and hydroids (*Tubularia* sp.). Additional organisms include algae/weeds (*Enteromorpha* and *Ectocarpus* sp.), polyzoans, tubeworms (serpulids), sponges (*Halichondria* sp.) and tunicates (sea squirts). The fouling challenge is seasonal and is characterised by a severe barnacle challenge from about May until about September.

Singapore test site

The Singapore test site is located in tropical coastal waters. Typical water temperatures range from about 25 - 30 ^{o}C with little seasonal variation. Salinity is generally about 35 % and sea water pH is generally within the range 8.00-8.25.

Fouling is characterised by an extremely heavy challenge from barnacles, molluscs, tubeworms, polyzoa, slime and felt which persists throughout the year with only slight seasonality.

Fouling of the test panels is assessed visually by the amount and type of each organism. Six main types of fouling are recognised: slime; adherent slime; brown felt; weed; barnacles; hydroids.

For each of these fouling types a subjective visual assessment is made and scored on a scale ranging from 0 to 3 in steps of 0.5. The scoring system is shown in Table 8.3.2.

Table 4.2 Subjective visual assessment scoring system

Subjective fouling	Fouling score
Nil	0
Trace	0.5
Slight	1
Slight to moderate	1.5
Moderate	2
Moderate to heavy	2.5
Heavy	3

Each visual score is then converted to a numerical rating by multiplication by an appropriate factor that is dependent on the type and importance attached to the differing fouling types. The factors are outlined below in Table 4.3. In completing an assessment the numerical ratings for each fouling type are added together and the result subtracted from 100 to give an overall antifouling rating for the coating under test. A completely clean panel would give an antifouling rating of 100 and a completely fouled panel an antifouling rating of 0.

Table 4.3 Weighting factors applied to the different types of fouling

Type of Fouling	Weighting or multiplication Factor
Slime	8
Adherent Slime	24
Brown Felt	24
Weed	32
Barnacles	32
Hydroids	32
Tubeworms	32
Bryozoans	32

Full details of scoring and assessment are provided for each fouling type. Photographs of the test panels taken at the end of the study are also provided to HSE.

SECTION 3: RAFT TEST METHOD 3

Full details of the study protocols have not been provided. Panels measuring 200 x 400 mm are coated with the test product and immersed in sea water on a raft for a period of time. A blank test panel serves as a negative control. Fouling of the panels is assessed periodically.

Singapore test site

The study site in Singapore is considered representative of tropical waters, with temperatures of 25 to 30 ^{o}C, and heavy fouling.

Norwegian test site

The Norwegian test site is considered representative of temperate waters with temperatures of 0 - 20 ^{0}C and a broad spectrum of fouling organisms.

UK test site

This is considered representative of temperate waters, with temperatures of 0 to 20 ^{o}C, and broad spectrum fouling. Because of the presence of excess nutrients (pollution), algal fouling is reported as heavy.

USA test site

This is considered representative for 'tropical' waters, with temperatures of 25 to 30 ^{o}C, and heavy fouling.

The fouling is classified as follows:

Light slime - bacteria, microalgae and protozoans that were easily removed from the test panel.

Dense slime - the above organisms, but removal from the panel was not easy.

Weed - green, red and brown algae.

Animals - barnacles, tubeworms, mussels, hydroids and bryozoa.

The extent of fouling, i.e. trace, slight, medium or heavy, is given a rating according to the type and degree of fouling. These weightings are presented in Table 4.4.

Table 4.4 Weighting system used by the company for assessing the degree of fouling in raft tests

Fouling organisms	Assessment (%)			
	Trace	Slight	Medium	Heavy
Light slime	0	1	3	5
Dense slime	3	5	10	20
Weed	5	10	30	50
Animals	5	10	30	50

The amount of fouling is calculated by adding up the weightings and then subtracting from 100. Zero growth then gives a rating of 100 (100-0), and heavy fouling a rating of 0 (100-100). The antifouling performance is evaluated as shown in Table 4.5.

Table 4.5 Antifouling performance rating

Performance	Rating (%)
Bad	0-50
Poor	50-70
Good	70-90
Very good	90-98
Excellent	98-100