Scientific Committee on Consumer Safety

SCCS

OPINION ON

Phenoxyethanol

The SCCS adopted this opinion at its 2nd plenary meeting

on 6 October 2016

About the Scientific Committees

Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) The Scientific Committees review and evaluate relevant scientific data and assess potential risks. Each Committee has top independent scientists from all over the world who are committed to work in the public interest.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee, on request of Commission services, provides Opinions on questions concerning health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (e.g. cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (e.g.: tattooing, artificial sun tanning, etc.).

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ISSN ISBN

Doi: ND-

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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

ACKNOWLEDGMENTS

SCCS members listed below are acknowledged for their valuable contribution to the finalisation of this Opinion.

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The additional contribution for the drafting of the preliminary Opinion of former members of the SCCS (2013-2016) and external experts listed below is gratefully acknowledged.

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This Opinion has been subject to a commenting period of a minimum four weeks after its initial publication. Comments received during this time were considered by the SCCS and discussed in the subsequent plenary meetings.
For this Opinion, comments received resulted in the following changes: *PBPK and toxicokinetic sections, including the respective parts in the discussion, as well as a corrective action on the last page of the opinion (Annex I) where the wrong part of the figure has been replaced.*

Keywords: SCCS, scientific opinion, Phenoxyethanol, Regulation 1223/2009, CAS No. 122-99-6, EC No. 204-589-7

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Phenoxyethanol, 16 March 2016, final version of 6 October 2016, SCCS/1575/16

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1. BACKGROUND

Phenoxyethanol CAS n. 122-99-6 as preservative is regulated in Annex V/29 of the Cosmetics Regulation (EC) n. 1223/2009.

According to the Cosmetics Regulation (EC) n.1223/2009 Phenoxyethanol is authorized as a preservative in cosmetic formulations at a maximum concentration of 1.0%.

In September 2012, the Commission received a risk assessment submitted by the French Agency ANSM (Agence nationale de sécurité des médicaments et des produits de santé) which rose concerns about the use of Phenoxyethanol as preservatives in cosmetic products.

The ANSM report (Evaluation du risque lié à l'utilisation du phénoxyéthanol dans les produits cosmétiques) concludes that the maximum authorised concentration (currently of 1%) of Phenoxyethanol for use as a preservative should be lowered to 0.4% in cosmetic products for children less than three years. In addition, Phenoxyethanol should not be used in cosmetic products intended for their nappy area. The Commission received information from other member States which raised similar concern on the use of Phenoxyethanol, in particular on children.

In December 2013, in response to a call for data on Phenoxyethanol by the Commission, Cosmetics Europe submitted a safety dossier in order to defend the current use of Phenoxyethanol as preservative in cosmetic formulations at a maximum concentration of 1.0%.

In December 2014, additional information from Cosmetics Europe (Subm. II) was received by the Commission and in July 2015 the submission of data was complemented with a safety assessment tool, such as the physiologically based pharmacokinetic (PBPK) modelling, in order to provide a perspective on systemic exposure of phenoxyethanol in humans (absorption, distribution, metabolism and excretion).

2. TERMS OF REFERENCE

1. *Does SCCS consider Phenoxyethanol safe for use as a preservative with a maximum concentration of 1.0 %, taking into account the information provided?*

2. *The SCCS is asked, when making the assessment, to take into account the specific age groups who might be particularly susceptible to the effects of Phenoxyethanol used as a preservatives in cosmetic products.*

3. *Does the SCCS have any further scientific concerns with regard to the use of Phenoxyethanol in cosmetic products?*

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

Primary name and/or INCI name

Phenoxyethanol

Chemical names

IUPAC name: 2-phenoxyethanol
Synonyms: Ethyene glycol monophenyl ether, phenoxytol, 1-hydroxy-2-phenoxyethane, (2-hydroxyethoxy) benzene

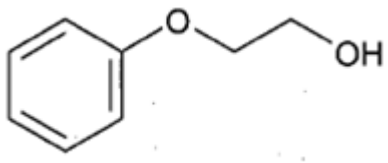
Trade names and abbreviations

Trade names:
Protectol® PE,
DOWANOLTM EPh (ELP),
NEOLONETM PH 100 Preservative
Abbreviations: EGPhE

CAS / EC number

CAS No.: 122-99-6
EC No.: 204-589-7

Structural formula



Empirical formula

$C_8H_{10}O_2$

3.1.2 Physical form

Oily, slightly viscous liquid at room temperature

3.1.3 Molecular weight

Molecular weight: 138.17 g/mol

3.1.4 Purity, composition and substance codes

Purity of ingredient as used in cosmetic products:
GC, quantitative: >99.5%
Water content: <0.1%

Batches used in toxicological evaluations:

Batch Code	Purity
82/125	80%
2219-93	No data
83/143	No data
66-4276	99.9%
3381-238	99.45%

N0119	99.9%
S14022T01	>95%
53 (C44172) – also referred to as 53	99.9%
C093082 / 01	94-95%
41183068EO	>99.9%
664287	99.9%
QF1750UKAO	99.7%

SCCS comment
Batch S14022T01 is probably batch S16022T01 in the 90-day repeated dose toxicity study (ref. 11).

3.1.5 Impurities / accompanying contaminants

Potential impurities

Phenol	<10 ppm
Ethylene oxide	<2 ppm

3.1.6 Solubility

In water at 20ºC, pH 7	25 g/L
In n-Hexane at 19.5°C ± 0.6°C	11.3g/L

Miscible with Methanol in a 1:1 (w/w) mixture at 19.4°C ± 0.6°C

3.1.7 Partition coefficient (Log P_{ow})

Log P_{ow}: 1.2 at 23 ºC, pH 7

3.1.8 Additional physical and chemical specifications

Organoleptic properties: Colourless in appearance; faint characteristic odour

Melting point:	9.1°C at 1013 hPa
Boiling point:	244.3°C
Flash point:	126°C at 1013 hPa
Vapour pressure:	0.01 hPa at 20°C; 0.18 hPa at 50°C
Density:	1.11 at 20°C
Viscosity:	41 mPa*s at 19.8°C
Refractive index:	1.535-1.539 at 25°C
pKa:	/
pH:	/
UV-Vis spectrum	(….. nm): /

3.1.9 Homogeneity and Stability

General Comments to physico-chemical characterisation

UV-Vis data was not provided.
Further data on identity and physico-chemical properties can be found in a recent review (ref. 105)

3.2 Function and uses

2-Phenoxyethanol is used as a preservative in cosmetic formulations at a maximum concentration of 1.0%.
2-Phenoxyethanol is a broad spectrum preservative which has excellent activity against a wide range of Gram negative and Gram positive bacteria, yeast and mould. It is also used as a solvent and, because of its properties as a solvent, it is used in many blends and mixtures with other preservatives.

2-Phenoxyethanol is not registered as a food additive in the EU.
Scognamiglio et al. (ref. 105) reported that 2-phenoxyethanol is a fragrance ingredient used in many fragrance mixtures (see discussion). An ester of 2-Phenoxyethanol, 2-Phenoxyethyl isobutyrate and 2-Phenoxyacetic acid, the main metabolite of 2-Phenoxyethanol, were mentioned in a WHO publication where 43 flavouring agents in food were evaluated (WHO 2003, AR4), however at intakes assessed to be very low in Europe (around 1 µg/kg bw/day).

3.3 Toxicological Evaluation

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

Guideline:	OECD Guideline no. 401 (1981)
Species/strain:	Rat/Wistar
Group size:	Five per sex per dose
Test substance:	2-phenoxyethanol
Batch:	82/135
Purity:	ca. 80% (techn)
Dose level:	681, 1470, 3160, and 5000 mg/kg bw
Vehicle:	0.5% Carboxymethylcellulose
Dose route:	Oral, gavage
Dosing schedule:	Single application
Observation period:	14 days
GLP:	/
Report date:	1982

Results
All animals dosed with 681 mg/kg bw survived the 14-day observation period. All males survived a treatment of 1470 mg/kg bw, whereas 2 out of 5 female rats died. At a dose level of 3160 mg/kg bw, 2 males and 4 females died, respectively. At the highest dose level 3 males and all females died. In all treatment groups, the latest time point where death occurred was observation day 1. Generally females were more susceptible to 2-phenoxyethanol than males.
Clinical signs included dyspnoea, apathy, abnormal posture, staggering, atony, deficiency in pain and cornea reflex, coma-like state, spastic gait, rough fur, exsiccosis, exophthalmoses, and general poor condition.
Animals that died during the study course revealed the following signs at necropsy: hyperaemic congestion; lungs which were slightly inflated; sporadically reddened glandular stomach.

Conclusion
The LD50 values in females and males were determined to be 1840 mg/kg bw and 4070 mg/kg bw, respectively. The LD50 value for males and females combined was determined to be 2740 mg/kg bw.

Study Reliability
Although this study was not conducted under GLP and the concentration of the test substance was reported as ca. 80%, the study is considered sufficiently reliable for evaluation of the endpoint of acute oral toxicity.

Ref.: 1

Two other acute oral toxicity studies in rats and rabbits, which dated from 1980 and were poorly documented, resulted in LD-50 values of 1- 2 g/kg bw.

Ref.: 2

3.3.1.2 Acute dermal toxicity

Guideline:	Draft IRLG (Interagency Regulatory Liaison Group) Guidelines for Selected Acute Toxicity Tests (August 1979)
Species/strain:	Rabbits/New Zealand White
Group size:	Four animals (2M, 2F)
Test substance:	Emeressence 1160 (2-phenoxyethanol)
Batch:	2219-93
Purity:	Not given
Dose level:	Limit dose of 2 mL/kg bw, corresponding to 2214 mg/kg bw
Vehicle:	Undiluted
Dose route:	Dermal, occluded
Dosing schedule:	Single application
Observation period:	14 days
GLP:	/
Report date:	1980

The dorsal skin surface of four rabbits was shaved 24 hrs prior to dosing and abraded within 2 hrs prior to dosing. Undiluted 2-phenoxyethanol was applied at a dose of 2 mL/kg bw under occlusive conditions and left in place for 24 hrs. At the end of the 24-hr exposure period, any unabsorbed material remaining on the skin was removed. Animals were observed for gross signs of systemic toxicity and dermal irritation. Examinations were carried out twice daily for a period of 14 days following completion of the exposure period. At the end of the 14 day observation period, rabbits were weighed and sacrificed and a gross necropsy was performed.

Results
No mortalities occurred in this study. Gross necropsy revealed no gross pathological findings in any of the major organs evaluated including heart, lungs, brain, gastrointestinal tract, liver, and spleen. Local erythema at the site of application was observed in two rabbits and was reversible within 3 days after dosing. Desquamation was observed at the site of application in one rabbit from days 3-14 after dosing. The LD50 for acute dermal toxicity in this study was >2214 mg/kg bw.
Although this study was not conducted under GLP and the test substance purity was not reported, the study is considered sufficiently reliable for evaluation of the endpoint of acute dermal toxicity.

Ref.: 2

SCCS comment
Lack of any gross pathological alterations or lesions is reported in all standard organs following dermal application, however, these were not investigated in the kidney.

3.3.1.3 Acute inhalation toxicity

No data available.

3.3.1.4 Acute intraperitoneal toxicity

No data available.

3.3.2 Irritation and corrosivity

Skin irritation

Guideline:	Similar to OECD Guideline no. 404 (1981)
Species/strain:	Vienna White rabbit
Group size:	Three (one male and two females)
Test substance:	Technical 2-phenoxyethanol
Batch:	83/143
Purity:	No data
Dose level:	0.5 ml, undiluted
Dose route:	Shaved skin, occlusive conditions
Dosing schedule:	Single application
Observation period:	72 hours
GLP:	No
Study period:	June 1983

0.5 ml of the test substance was applied for a 4-hour exposure period onto the intact skin of each of 3 White Vienna rabbits to an area of 2.5 cm². After the occlusive exposure, the patch was removed and the application site was washed with water/Lutrol (1:1). The animals were observed for 72 hours; the skin sites were scored at 30-60 minutes after removal of the patch, and at 24, 48 and 72 hours after the beginning of the exposure.

Results
Two of the three animals had an erythema score of 1 at the 4-hour time point. The erythema was reversible within 24 hours and erythema scores for all three animals were 0 at 24, 48, and 72 hours. Oedema scores for all three animals were 0 at all time points.

Conclusion
Undiluted 2-phenoxyethanol was not irritating to rabbit skin under the conditions of this study.

Ref.: 4

SCCS comment
The scores of skin reactions were performed at 30-60 minutes after removal of the occlusive dressing, but in the tabular summary, the reactions were presented as scored at 4 hours after removal of the occlusive dressing.
Under the conditions of this study, the undiluted test substance is considered as a mild irritant to the rabbit skin.

Mucous membrane irritation / Eye irritation

Guideline:	Similar to OECD Guideline no. 405 (1981)
Species/strain:	Vienna White rabbit
Group size:	Three animals (one male and two females)
Test substance:	Technical 2-phenoxyethanol
Batch:	83/143
Purity:	No data
Dose level:	0.1 ml, undiluted
Observation period:	15 days
GLP:	No
Study period:	June 1983

0.1 ml of the undiluted test substance was placed into the conjunctival sac of the right eye of each of 3 Vienna White rabbits. The test substance was not washed out. The untreated eye served as control. The readings were performed at 1, 24, 48 and 72 hours, as well as 8 and 15 days after instillation of the test substance.

Results
Undiluted 2-phenoxyethanol produced clear signs of eye irritation in all three animals. Redness (score 2) and swelling (score 2) of the conjunctiva, as well as secretion (score 1 or 2) was observed in all three animals at 1 hour; redness (score 2) was still observed in two animals at 72 hours and swelling (score 1) in one animal. The iris was affected in all animals at 24 and 48 hours (score 1), in two animals at 72 hours (score 1 and 2), and in one animal at 8 days (score 1). Corneal opacity was observed in all animals at 24, 48, 72 hours and 8 days (score 1 or 2) and in one animal at 15 days (score 1); the whole corneal area was affected at 24, 48 and 72 hours, half of the area at 8 days and one fourth of the area at 15 days. Irritation was of maximal severity between 48 and 72 hours following application. Thereafter, the irritation subsided and after 15 days, only one animal still displayed slight corneal opacity affecting less than one fourth of the corneal area of the treated eye.

Conclusion
Under the conditions of this test, 2-phenoxyethanol was irritating to the eye.

Ref.: 5

SCCS comment
Under the conditions of this study, the undiluted test substance is an irritant to the rabbit eye.

3.3.3 Skin sensitisation

Guinea Pig Maximisation Test

Study design:	
Guideline:	According to OECD guideline no. 406
Species:	Guinea pig/Hsd Poc:DH (SPF)
Group size:	10 females per dose
Test substance:	Protectol® PE (2-Phenoxyethanol)
Batch:	66-4276
Purity:	99.9 area% (HPLC?)
Intradermal induction:	0.1% in olive oil Ph.Eur./DAB or 0.1% in Freund's adjuvant (FCA)/0.9% saline (1:1)
Epicutaneous induction:	1 ml undiluted test substance
Challenge:	0.5 ml undiluted test substance, epicutaneous application under occlusive conditions
Positive Control:	Hexyl cinnamic aldehyde, 85%
GLP:	Yes
Study Period:	06 Dec 2001 – 27 Feb 2002
Report Date:	2002

A pre-test was performed using intradermal injections of test substance (5%, 1% and 0.1%) in olive oil Ph. Eur./DAB or in 1:1 Freund's adjuvant (FCA) in 0.9% saline). Reactions were assessed after 24 and 48 hours. Necrotic skin lesions were induced by 5% and 1% solutions of the test substance. For the main study, 0.1% of the test substance in 1:1 with FCA was used for the intradermal induction. Another pre-test showed that the undiluted test substance could be used for epicutaneous induction.

In the main study, animals received intradermal injections with 0.1 %.

A. Induction Exposure
In the main study, animals (n=10) in the test group received three pairs of intradermal injections in the neck region (saline FCA alone: 0.1% test substance in olive oil, and 0.1% test substance in saline FCA). Control animals were treated in the same way, but without the test substance. Epicutaneous induction was carried out one week later in the same region by applying 1 ml undiluted test substance to the skin under occlusive dressing for 48 hrs.

B. Challenge Exposure
Control and test animals were challenged two weeks after epicutaneous induction on the flank with the undiluted test substance.
Skin reactions were assessed according to the grading scale of Magnusson and Kligman.

Results
Treatment had no impact on body weight gain and no signs of toxicity were seen. After the intradermal induction, intense erythema and swelling were observed at the injection sites of all control group animals and all test group animals to which only FCA in saline was applied. At the injection sites of the 0.1% test substance preparation in FCA (1:1), intense erythema and swelling were seen in all test group animals.
Injections of a 0.1% test substance preparation in olive oil caused moderate and confluent erythema and swelling in all test group animals.
The control group animals injected with olive oil showed moderate and confluent erythema and swelling. A 50% formulation of olive oil with FCA in saline (1:1) caused intense erythema and swelling in all control group animals.
The epicutaneous induction with the undiluted test substance led to incrustation and moderate and confluent erythema and swelling in all test group animals.
The challenge with the undiluted test substance on the flanks did not cause any skin reactions in either the control or test group animals.

Conclusion
Under the experimental conditions of this study, 2-phenoxyethanol was not a skin sensitiser.

Ref.: 6

SCCS comment
No analytical report on the purity of batch 66-4276 is available.

3.3.4 Dermal / percutaneous absorption

Percutaneous absorption *in vitro*

First study:

Guideline:	/
Tissue:	Wistar rat (dorsal skin) and human donors (breast, leg, abdominal skin from surgery)
Method:	Static (0.79 cm^2 exposure area) or flow-through (0.64 cm^2 exposure area) diffusion chambers
No. of chambers:	Skin from 7 rats (5 cells/rat) for static diffusion cells (uncovered conditions); skin from 6 rats for static diffusion cells (covered conditions); skin from 6 rats for flow-through diffusion cells; skin from 3 human donors for flow-through conditions (uncovered conditions)
Test substance:	2-phenoxyethanol
Batch:	purchased from Sigma, no batch information available
Purity:	Not reported

Test substance:	2-Phenoxy-[1-^{14}C]ethanol
Batch no:	No information available
Radiochemical purity:	>99%
Concentrations:	1556 nmol (0.81 µCi) in 10 µl methanol for static diffusion cell; 5290 nmol (2.73 µCi) in 10 µl methanol for flow-through diffusion cell
Vehicle:	Methanol
Receptor fluid:	50% v/v ethanol/water (static conditions) or modified Earle's medium (flow through conditions)
GLP:	No information available
Publication date:	1997

In this study, the absorption and metabolism through rat and human skin *in vitro* was determined and dermal metabolism was assessed with post-mitochondrial fractions from rat skin homogenates.

Dermal absorption was evaluated using static and flow-through diffusion cells (rat skin) or flow-through diffusion cells (human skin). Radiolabelled 2-phenoxyethanol was applied to the surface of the skin in the diffusion cell in 10 µl methanol. For the rat skin studies, the calculated amounts applied were 272 µg/cm^2 for the static cell experiments and 1140 µg/cm^2 for the flow-through cell experiment. For the study with human skin, the calculated amount applied was 552 µg/cm^2 in a flow-through cell experiment. For the static cell experiments, the receptor fluid was 50% v/v ethanol/water. For the flow-through cell experiments, the receptor fluid was modified Earle's medium. Radioactivity in surface wash, stratum corneum, epidermis and dermis, and receptor fluid was measured. Receptor fluid was sampled over 24 hours in the experiments with rat skin and over 6 hours in the experiment with human skin (due to the limited supply of human skin).

The time course of appearance of 2-phenoxyethanol in receptor fluid was measured by HPLC. Receptor fluid was also analysed for 2-phenoxyacetic acid but none was detectable.

Results
In rat skin under uncovered conditions with static cells, 64 ± 4.4% of the applied dose was absorbed through the skin after 24 hours. Only 0.9% was associated with the stratum corneum and 1% was recovered in skin (epidermis and dermis) at 24 hours. The total recovery was only 67.6%. The low recovery was attributed to evaporative loss from the skin surface under uncovered conditions. When absorption in rat skin was evaluated under covered conditions with static diffusion cells, the recovery was 102.6%, and 98.8±7.0% was found in the receptor fluid. In this experiment, absorption reached a plateau within 4 hours. With flow-through diffusion cells, 43±3.7% of the applied dose was absorbed through the skin after 24 hours. The amounts in the stratum corneum and skin layers (epidermis and dermis) at 24 hours were 2.9% and 1.6%, respectively. Recovery in this experiment was only 51% and the low recovery was expected to be due to evaporation.

In skin from human donors, 59.3±7.0% of the applied radioactivity was absorbed through the skin and recovered in receptor fluid after 6 hours. Similar absorption profiles were observed for the three donors. The stratum corneum and epidermis/dermis contained 4.1 and 4.4% of the dose, respectively. No data on evaporative losses were reported.

Ref.: 9

SCCS comment
The study description was from a report publicly available. The study is considered to have deficiencies (only 3 human skin donors, poor recovery, only a 6-hour absorption period for human skin experiments). Another major shortcoming of the study is the use of methanol as solvent, which may act as an absorption enhancer and may also compete with 2-phenoxyethanol for oxidation by dehydrogenases in skin, thereby potentially confounding the test results. The study is considered of limited value.

Second study:

Guideline: According to OECD guideline no. 428

Tissue:	Human skin (collected from the abdomen during plastic surgery, frozen stored tissue) (N=10 per formulation and per 2-phenoxyethanol concentration, from 5 donors)
No. of chambers:	10 cells per formulation and 2-phenoxyethanol concentration (2 cells per formulation and concentration for each of 5 donors)
Method:	Franz™ static diffusion chambers
Test substance:	2-phenoxyethanol
Batch:	3381-238
Purity:	99.45%
Test item:	2-Phenoxyethanol [ethanol-1,2-^{14}C]
Source:	New England Nuclear, Boston, USA
Chemical purity:	>97.5%
Vehicle:	Cleaning gel and body lotion formulations (each containing either 0.2 or 1% 2-phenoxyethanol)
Concentrations:	Formulations containing 0.2% 2-phenoxyethanol: mean amount of formulation 4.60 mg/cm^2, corresponding to 9.20 ± 1.09 µg 2-phenoxyethanol/cm^2. Formulations containing 1% 2-phenoxyethanol: mean amount of formulation 5.33 mg/cm^2, corresponding to 54 ± 5 µg 2-phenoxyethanol/cm^2
Receptor fluid:	phosphate-buffered saline, pH 7.4
GLP:	/
Study period:	26 Mar 2002 – 25 Apr 2002
Report date:	2002

Percutaneous absorption of 2-phenoxyethanol (PE) was evaluated in a typical cleaning gel (a rinse-off formulation) and body lotion (a leave-on formulation) formulations containing either 0.2% or 1% 2-phenoxyethanol. Human skin samples (obtained from surgery) were mounted in Franz™ static diffusion cells (surface area 1.76 cm^2) and maintained at a temperature of 32ºC. For each diffusion cell, the integrity of the skin barrier was verified before application of the study formulation by measuring the trans-epidermal loss of water.
After checking skin integrity (all cells were acceptable), the different formulations were applied and each cell was covered with a semi-occlusive filter (MICROPORE™, Scotch 3M). For the rinse-off experiments, the cleaning gel formulation was washed from the skin surface at 30 min. For the leave-on experiments, the body lotion formulation remained on the skin until the last sampling point at 24 hrs. The lower compartment was filled with phosphate buffered saline as receptor fluid, and the receptor fluid was completely collected at 3, 6, 9, and 12 hrs and replaced by fresh fluid and at the last sampling point of 24 hrs. At the end of the 24-hr period, the cells were dismantled and the skin was kept to determine the amount of radiolabel present in the tissue. Because of the fragility of the skin (skin biopsies were kept frozen before their use), the stratum corneum was not removed from the epidermis by tape stripping. The epidermis with stratum corneum was separated from the dermis using forceps. The amount of radiolabel present in skin compartments, receptor fluid and rinsing solution was measured by liquid scintillation counting. A mass balance was calculated.

Results
The results of the study are summarised in Tables 1 and 2.

Table 1
Rinse-Off Formulation

	Receptor Fluid (RF)	Epidermis +Stratum Corneum (E+SC)	Dermis (D)	Total (SC+E+ D+RF)	Rinsing Soln.	Semi-occlusive Filter	Mass Balance (% applied dose)
Rinse-off Cleaning Gel – Expressed as $\mu g_{eq}/cm^2$ (mean ± SD)							
1% PE	19.97±4.94	0.068±0.017	0.115±0.054	20 ± 5	28 ± 7	1.06±0.21	-
0.2% PE	3.16±0.75	0.014±0.005	0.018±0.008	3 ± 1	4.76 ± 0.89	0.19±0.02	-
Rinse-off Cleaning Gel – Expressed as % of applied dose (mean ± SD)							
1% PE	36 ± 10	0.12 ± 0.03	0.21 ± 0.10	37 ± 10	50±10	1.91±0.40	88.59 ±6.38
0.2% PE	33 ± 8	0.14 ± 0.04	0.18 ± 0.08	34 ± 8	50 ± 7	2.03±0.30	86.47 ±3.67

Table 2
Leave-On Formulation

	Receptor Fluid (RF)	Epidermis + Stratum Corneum (E+SC)	Dermis (D)	Total (SC+E+D + RF)	Rinsing Solution	Semi-occlusive Filter	Mass Balance (% applied dose)
Leave-on Body Lotion – Expressed as $\mu g_{eq}/cm^2$ (mean ± SD)							
1% PE	40.17±4.19	0.14±0.04	0.25±0.12	41 ± 4	0.856±0.162	4.12±1.59	-
0.2% PE	7.25±1.75	0.02±0.01	0.05±0.01	7 ± 2	0.086±0.024	1.00±0.36	-
Leave-on Body Lotion – Expressed as % of applied dose (mean ± SD)							
1% PE	77.66±7.48	0.26±0.06	0.49±0.23	78 ± 7	1.653±0.300	7.97±3.12	88.65±5.95
0.2% PE	80.32±10.32	0.19±0.05	0.52±0.11	81 ± 10	0.978±0.310	11.40±4.33	99.00±6.49

After 30-min exposure with the rinse-off formulation, approximately 50% of the applied dose, independent of the applied concentration, remained on the skin surface and was removed by the washing procedure. The amount of radioactivity retained in the semi-occlusive filter was negligible (1.91 ± 0.40% and 2.03 ± 0.30% of the applied dose for the 1% and 0.2% concentrations, respectively).

After 24-hr exposure with leave-on formulation, the amount of radioactivity removed by the washing procedure was 1.65 ± 0.30% and 0.98 ± 0.31% of the applied dose for the 1% and 0.2% concentrations, respectively. The amount of radioactivity retained in the semi-occlusive filter was 7.97 ± 3.12% and 11.40 ± 4.33% of the applied dose for the 1% and 0.2% concentrations, respectively. The filter served as a trap for evaporative loss, which was more significant under the leave-on conditions than for the rinse-off conditions.

The mass balance for recovery of radioactivity in the experiments with the rinse-off formulation was 88.59 ± 6.38 and 86.47 ± 3.67 of the applied dose for the 1% and 0.2% concentrations, respectively. For the leave-on formulations, the mass balance was 88.65 ± 5.95 and 99.00 ± 6.49 of the applied dose for the 1% and 0.2% concentrations, respectively.

Note that in a special experiment conducted within this study, mass balance was evaluated for four diffusion cells (one cell for the rinse-off and leave-on formulations each containing either 1% or 0.2% 2-phenoxyethanol) under conditions of full occlusion with PARAFILM™ during the 30-min (rinse-off) or 24-hr (leave-on) exposure period. The mass balance of radioactivity in this special experiment ranged from 90 to 102% recovery for these four cells. This information supports the conclusion that the somewhat lower recovery in the main experiments (88.65 to 99.00%) can be attributable to losses due to volatility. For volatile test substances, the OECD GD28 guidance indicates that a mass balance of 80-120% is acceptable (*Ref 8*). Therefore the mass balance for the main experiments in this study is acceptable.

At the end of the 24-hr incubation period, the amount of radioactivity recovered in the skin was very small in comparison to the amount that was measured in the receptor fluid. For the rinse-off formulation, the percentage detected in the epidermis + stratum corneum was 0.12 ± 0.03% and 0.14 ± 0.04% for the 1% and 0.2% concentrations, respectively. For the leave-on formulation, the corresponding values for the epidermis + stratum corneum were 0.26 ± 0.06% and 0.19 ± 0.05% for the 1% and 0.2% concentrations, respectively. Amounts detected in the dermis were likewise very small, ranging from 0.18 ± 0.08% to 0.21 ± 0.10% for the rinse-off formulation and 0.49 ± 0.23% to 0.51 ± 0.11% for the leave-on formulations. These results indicate that there is no binding or accumulation of 2-phenoxyethanol in the skin.

For both the rinse-off and leave-on formulations, diffusion of 2-phenoxyethanol into the receptor fluid was rapid, with a maximum flux observed at the end of the 3-hr time point. For the rinse-off formulation, the percentage appearing in the receptor fluid by the end of the 24-hr period was 37 ± 10% and 34 ± 8% for the 1% and 0.2% concentrations, respectively. For the leave-on formulation the corresponding values were 78 ± 7% and 81 ± 10% for the 1% and 0.2% concentrations, respectively.

Although the amount found in the stratum corneum is typically excluded when calculating the amount dermally absorbed, in this study the stratum corneum was not removed from the epidermis. Therefore, the amount measured in the epidermis + stratum corneum, the dermis, and the receptor fluid (SC + E + D + RF) was summed to calculate the extent of dermal absorption. The amount absorbed through the skin expressed as a percentage of the applied dose for the rinse-off formulation was similar for the two concentrations studied (37 ± 10% and 34 ± 8% for the 1% and 0.2% concentrations, respectively). Likewise, for the leave-on formulation, the percentage absorbed was independent of the concentration (78 ± 7% and 81 ± 10% for the 1% and 0.2% concentrations, respectively).

Ref.: 7

SCCS comment

For MoS calculations, mean values + 1 SD may be used: 37% + 10%, i.e. **47%** dermal absorption for 1% 2 phenoxyethanol in rinse-off formulations and 78% + 7%, i.e., **85%** for 1% 2 Phenoxyethanol in leave-on formulations. Use of this data for the calculation of a systemic exposure dose (SED) is not necessary since calculation of the margin of safety is based on a dermal toxicity study.

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (10 days) oral toxicity

According to a published report, groups of three New Zealand White female rabbits weighing 3 -4.5 kg were administered 2-phenoxyethanol orally by gavage at 100, 300, 600, or 1000 mg/kg bw/day for 10 consecutive days. In addition, six female rabbits were administered distilled water (vehicle control) for a similar period. All rabbits received a single dose per day. Body weights were recorded on days 1, 5, and 10 of treatment. At necropsy, blood samples for haematological measurements samples for urinalysis and representative sections of organs for histopathological analysis were taken and preserved.

Results
During the 10-day treatment period, deaths occurred in all dose groups above 100 mg/kg bw/day or animals were sacrificed moribund. In the 600 and 1000 mg/kg bw/day dose group, none of the animals survived. In the 300 mg/kg bw/day dose group, one animal was found dead on day 10 after dosing. Signs of toxicity were characterised by anorexia, lethargy and excretion of dark-red urine. On day 5, mean body weights of rabbits dosed with 100, 300, or 600 mg/kg bw/day had decreased approximately 8% from their pre-exposure weights and 10-14% from that of control animals. On day 10, rabbits in the 100 and 300 mg/kg/day dose groups continued to show similar decreases in body weight. Control rabbits exhibited a slight gain in mean body weight over the 10-day dosing period.
In general, rabbits exposed to 2-phenoxyethanol had decreased RBC count, HGB concentration and PCV, with concurrent increases in nucleated and poly-chromatophilic red blood cells. Many animals exhibiting severe depressions in RBC numbers also showed concurrent increases in MCH, MCHC, platelets and WBC. The values for MCH and MCHC were considered elevated due to free haemoglobin in plasma.
Urine samples collected from rabbits in the 1000, 600 and 300 mg/kg/day dose groups exhibited decreased pH in combination with elevated levels of protein, bilirubin, urobilirubin and haemoglobin. Decreases in urinary pH and increases in urinary bilirubin and RBC were observed in at least one rabbit in the 100 mg/kg/day dose group.
Most rabbits administered 1000 or 600 mg/kg bw/day exhibited gross pathological alterations consistent with haemolytic anaemia including enlarged and dark kidneys and spleen, dark urine in the urinary bladder, and dark urine staining the perineal region. Conversely, rabbits gavaged with 100 or 300 mg/kg bw did not have treatment-related gross lesions.
Treatment-related microscopic changes were observed in rabbits from all dose levels. Splenic microscopic changes included red pulp congestion and erythro-phagocytosis in most animals of both high dose groups. Treatment-related microscopic changes also occurred in the kidneys and stomach of both high-dose groups. The spleen of one rabbit in the 300 mg/kg/day dose group had thrombi in venous sinuses resulting in generalised splenic necrosis. This animal also had thrombi within pulmonary blood vessels. Two rabbits given 100 mg/kg/day had splenic extramedullary haematopoiesis.

Ref.: 12

SCCS comment
In conclusion, in this study with rabbits orally exposed by gavage for 10 days, the **LOAEL** is **100 mg/kg bw day**.
For the understanding of this Opinion, it is important to note that haematotoxicity in rabbits is caused by the parent compound when systemically available but not by the main metabolite 2-phenoxy acetic acid, which may be responsible for toxicity to the kidney (see section Toxicokinetics 3.3.5 and Special Investigations 3.3.12).

3.3.5.2 Repeated Dose (14 days) inhalation toxicity

Guideline:	According to OECD Guideline 412
Species/strain:	Rat/Wistar
Group size:	Five per sex per dose
Test substance:	2-phenoxyethanol
Batch:	41183068E0
Purity:	> 99.9 core peak-area% (GC)
Dose level:	0, 40, 200, 1000 mg/m³ (nominal)
Vehicle:	air
Dose route:	Inhalation, nose/head only
Dosing schedule:	6 hours per day, 5 days per week for 2 weeks (10 exposures)
Observation period:	14 days
GLP:	Yes
Study period:	06 Sep 2005 - 17 Feb 2007
Report date:	2007

This study was conducted to characterise the toxicity profile of 2-phenoxyethanol including target organs and to determine a NOAEL after 2-weeks exposure to liquid aerosols. Special emphasis was given to potential irritation effects in the respiratory tract. A total of 10 Wistar rats (5 males and 5 females) per test group were head-nose exposed to liquid aerosols for 6 hours per day, 5 days per week for 14 days (10 exposures). The target concentrations were 40, 200, and 1000 mg/m³. The mean measured concentrations were 48.2, 246, and 1070 mg/m³. A concurrent control group was exposed to conditioned air. On exposure days, the clinical examination was performed before, during and after exposure. Body weight and food consumption were determined at the start of the exposure period, on day 7 and on day 13. At study termination, clinical-pathological examinations of the blood, gross necropsy, measurement of organ weights and histopathological examinations of selected organs were conducted (liver, kidneys, adrenals, testes, thymus, spleen, lung).

Results
Repeated inhalation exposure of rats to an aerosol containing up to 1000 mg/m³ of 2-phenoxyethanol for 6 hours per day for a total of 10 exposure days showed low inhalation toxicity. Decreased body weight gain (females only) and food consumption (males and females) were observed in animals exposed to 1000 mg/m³ of 2-phenoxyethanol. There were no treatment-related changes in clinical chemistry or haematology and no treatment related histopathological changes indicative of systemic toxicity. Morphological changes such as minimal to slight degeneration/squamous metaplasia, hyperplasia/hypertrophy and minimal to slight inflammatory cell infiltrates were noted, indicating irritation potential in the nasal cavity, larynx and lung in mid- and high-dose animals. Lung weights were increased in mid- and high-dose males.

Conclusion
The No-observed-adverse-effect concentration (NOAEC) for local effects in upper airways and lung was determined to be 48.2 mg/m³.

Ref.: 3

SCCS Comment
No data on lung lavage is available. The NOAEC derived should be multiplied with a factor of 5/7 in order to take into account the intermittent exposure (5 days a week). An **adjusted NOAEC** of **34.4 mg/m³/day** may be used.

3.3.5.3 Sub-chronic (90 days) toxicity (oral)

Study 1 (conducted 2002)

Guideline:	According to OECD Guideline no. 408

Species/strain:	Rat/Wistar – HsdCpb:WU
Group size:	10 per sex per dose (main and recovery groups); 5 animals per sex per dose (satellite and satellite recovery groups)
Test substance:	2-Phenoxyethanol
Batch:	N0119
Purity:	99.9%
Dose levels:	0, 500, 2500 and 10000 ppm in diet corresponding to 34.0, 169.0, and 697.0 mg/kg bw/day in males and 50.2, 233.8, and 938.8 mg/kg bw/day in females
Vehicle:	Diet
Route:	Oral, via diet
Exposure period:	90 days with a 4-week recovery period for high dose animals
GLP:	Yes
Study Period:	Oct 2001 - Feb 2002 (in-life phase)
Report Date:	Oct 2002

Control groups of males and females received diet alone. Recovery group animals were given diet containing 0 or 10000 ppm 2-phenoxyethanol for 90 days followed by a four-week recovery period. Additional groups were administered 0, 500, 2500, and 10000 ppm in diet for 13-14 weeks or 0 and 10000 ppm in diet as satellite treatment groups and satellite recovery groups, respectively. Animals in all satellite groups were subjected to whole body perfusion during necropsy for possible neuropathological investigations.

Results
Analytical data verified that the test material content in diet was within acceptable limits of the target concentrations (97-111%); adequate homogeneity was also verified.

In-life findings:
Survival was unaffected by treatment. There was no difference between untreated and treated animals in general behaviour up to and including 10000 ppm in diet. Food and water consumption and body weight increase in treated animals were comparable to controls throughout the study. The functional observational battery, locomotor activity and grip strength testing showed no treatment-related effects. Therefore, no further neuropathological examinations were performed in animals from the satellite groups.

Terminal examinations:
A statistically significant reduction in the mean corpuscular haemoglobin concentration (MCHC) was observed in males at 10,000 ppm and at all doses in females of the main group at week 13. These changes were marginal (<3% decrease relative to controls) and not dose-related. Therefore, they were not considered treatment-related. No difference in MCHC was observed in animals in the recovery groups. A statistically significant increase in mean corpuscular volume (MCV) was observed in females at 500 ppm and 10,000 ppm. These changes were also minimal (<4% decrease relative to controls), and there was no evidence of a dose-response. No difference in MCV was observed in recovery group animals. No changes were observed in any of the other red blood cell parameters in either males or females – haemoglobin (HgB), haematocrit (HCT), mean corpuscular haemoglobin (MCH) and reticulocytes (Retic).

Leucocyte counts and differential blood count showed no treatment-related changes in main and recovery groups. The statistically significant decreases observed for males in leucocytes and lymphocyte counts at 2,500 ppm and 10,000 ppm can be attributed to relatively high values in the control group and were not considered to be treatment-related.

Alanine aminotransferase (ALAT) was statistically significantly increased in males at 10,000 ppm but not in females. Since no histopathological changes in liver or other observations suggesting liver toxicity were observed in high-dose males, and no increase in ALAT or liver histopathology was observed in females, this observation was considered to be incidental.

At the end of the treatment period, plasma triglycerides (at 500 ppm in males) calcium (at 500 ppm in females), and chloride (at 10,000 ppm in females) were statistically significantly increased whereas cholesterol (at 10,000 ppm in females), protein (at 10,000 ppm in females) and albumin (at 10,000 ppm in females) were statistically significantly decreased. These statistically significant differences relative to controls were slight (<10%), showed no clear dose-response relationship, were predominantly in the range of historical values, and were therefore considered not to be treatment-related.

T4 was statistically significantly increased in females at 10,000 ppm. After the recovery period, T3 was statistically significantly increased in males and decreased in females at 10,000 ppm. However, all values were in the range of historical control values. In addition, deviations from control values were small and not consistent for both sexes. Therefore, they are considered to be of no biological relevance.

Determination of enzymatic activity in liver tissue samples showed statistically significant increases as well as decreases for N-demethylase at 2500 ppm, O-demethylase at ≥ 500 ppm and CYP at ≥ 2,500 ppm in males at the end of treatment. In females, statistically significant increases were observed at 10,000 ppm for O-demethylase and CYP. No effects on these parameters were seen in recovery group animals. These observations showed no dose-response and were predominantly in the range of historical controls. They can be considered to reflect induction of liver enzymes but they do not reflect liver injury.

At the end of the treatment period, some absolute organ weights were increased, but not in the highest dose group. Since there were no histopathological findings pointing to any treatment-related effect in these organs, these differences relative to the controls were considered not to be indicative of organ-related toxicity. There was no evidence of treatment-related effects on histopathology in any of the tissues evaluated. There were no treatment-related ophthalmological findings.

Conclusion
Treatment with 2-phenoxyethanol at concentrations of 500, 2,500 and 10,000 ppm in the diet showed no evidence of treatment-related adverse effects. The no-observed-adverse-effect level **(NOAEL**) for 2-phenoxyethanol was therefore concluded to be 10,000 ppm in diet corresponding to an intake of **697 mg/kg bw/day** in males and **939 mg/kg bw/day** in females.

Ref.: 10

SCCS comment
The SCCS noted that in both the control groups and in all treatment groups, parameters of food intake, water intake and body weight gain declined during the study and persisted during the recovery period. These unusual data are not commented upon and not explained. It is assumed that the animals of this study were ill, potentially due to an infection. The study is considered of limited value and not reliable.

Study 2 (conducted 1987)

Guideline:	According to OECD guideline no. 408 (1981)
Species/strain:	Rat/Colworth Wistar
Group size:	15 per sex per dose (main groups); 5 animals per sex per dose (recovery groups)
Test substance:	2-Phenoxyethanol
Batch:	S16022T01
Purity:	≥95% by GC and by ^{13}C NMR
Dose levels:	0, 0.05, 0.1, 0.2, and 0.5% in diet corresponding to 0, 40, 81, 164, and 419 mg/kg bw/day (mean combined male and female intake)
Vehicle:	Diet

Route:	Oral, via diet
Exposure period:	90 days with a 5-week recovery period for high dose animals
GLP:	No (see further information below)
In Life Period:	17 Aug 1987 – 21 Dec 1987
Report Date:	Summary report produced in 1993 (see further information below)

In the study summary report of 1993, a dose range-finding study (4 weeks treatment) was mentioned with dosing of 0.00%, 0.05%, 0.10%, 0.20% and 0.50% of the test substance in the diet corresponding to about 0, 50, 100, 200 and 500 mg/kg bw/day. Some minor effects were observed at the highest dose, which were not considered adverse (no details described). Therefore, a NOAEL at the dietary level of 0.50% corresponding to 550 mg/kg bw/day was concluded.

Groups of 15 male and 15 female Colworth Wistar rats were fed 2-phenoxyethanol in the diet at levels of 0.05, 0.1, 0.2 or 0.5% for a period of 13 weeks. A similarly constituted control group received the untreated diet for the same period. An additional 5 male and 5 female recovery group rats were included in the control and high dose groups, and after the 13-week treatment period were maintained on the untreated control diet for a further 5 weeks to assess the reversibility of any changes seen.

Results
Homogeneity and stability of 2-phenoxyethanol in the test diet was assessed in a previous study. Diets in this 13-week study were analysed on three occasions during the study for concentration of 2-phenoxyethanol and were within acceptable limits.

In-life findings:
There were no unscheduled deaths and no clinical signs attributable to treatment. There were no clear effects on body weight, body weight gain or food intake. The study director commented that statistically significant higher body weights were observed for females at the low dose (0.05%) when compared with controls. There were, however, no apparent body weight changes at the higher doses. Statistically significant lower food efficiencies were observed over the 13-week treatment period for male rats at 0.5%. This was also apparent in males during the 5-week recovery period. Higher water intakes were seen during the treatment period for female rats at 0.5%. This was not apparent during the recovery phase.

During week 11, higher urine gamma-glutamyltransferase and urine alanine aminopeptidase values were noted for males at 0.5%. Urinalysis was therefore repeated for male rats at week 12. The findings were neither seen at this repeat analysis nor during the recovery phase.

Terminal examinations:
Absolute liver weights decreased by around 7-8% at the dose levels 0.2 and 0.5%. There were no other consistent organ weight differences that suggested an effect of treatment.
Small changes in clinical chemistry and haematology parameters are indicated below:
1. Compared with the control group, males dosed with 0.5% in diet showed increased plasma alkaline phosphatase (332.7 U/l compared with 298.7 U/l in controls). Other plasma enzymes (aspartate transaminase, alanine transaminase, lactate dehydrogenase, 1-hydroxybutyrate dehydrogenase, creatine kinase, pseudo-cholinesterase, and 5-nucleotidase) in male rats were not significantly different when compared to controls. In female rats, no increase in alkaline phosphatase was observed. The only change in plasma enzymes observed was a small but statistically significant decrease in plasma pseudo-cholinesterase observed at 0.05%, 0.2% and 0.5% in diet (-9% to -12% but no dose-response relationship). These small differences were not seen in recovery group animals.
2. Plasma total cholesterol was lower than the control values for male rats in the highest dose group (-11%) and for female rats in the dose groups 0.2% and 0.5% in diet (-7% and

-9%, respectively). A statistically significant decrease (-8%) was observed for the recovery phase of high dose male rats at week 18.
3. A marginal but statistically significant decrease in serum protein (-4%) was seen in male rats at 0.5% in diet. No change was seen in the recovery group animals. No effect on serum protein was observed in female rats.
4. In males at 0.2 or 0.5% in diet, marginally reduced serum beta globulin levels (both -4%) were seen compared with controls, and in males at 0.5% in diet there were slightly increased albumin levels and A/G ratio. These differences were not apparent after the recovery period. No changes in serum beta globulin, albumin or A/G ratio were observed in females.
5. At all doses, platelet counts were statistically lower than controls for one or both sexes (males -4% to -8%; females -8% to -10%). Pooled data of both sexes gave a dose-response relationship with reduced values of -5% to -8% compared with controls. Values of all dose groups normalised during the recovery phase, whereas an increase of 5.5% was noted for females at 0.5% in the diet.
6. In females, statistically significant increases of 2% both in haemoglobin and haematocrit were observed in the dose group of 0.2% in diet. These are considered to be of no toxicological significance. A marginal but statistically significant increase in MCV (+1%) was seen in the dose group 0.5% in diet. No changes in other red blood cell parameters were observed in females at the high dose. In males no statistically significant changes in the red blood cell parameters (HgB, HCT, MCV, MCH, and MCHC) were observed. Therefore the marginal increase in MCV in high-dose females can be considered an incidental finding.
7. Activated partial thromboplastin time was marginally increased by around 1 second compared with control for females at 0.5% at the end of the treatment period but not after the recovery period.

There were no macroscopic changes that were considered treatment-related. The only histopathological change related to treatment was a reduced level of total parenchymal lipid deposition in male rats at 0.5% (observed following Oil red O staining). This was not apparent at the recovery sacrifice. The study authors judged that this change was not of toxicological significance.

Conclusion
The study director set the no-observed-effect level (**NOEL**) at the dietary level of 0.2%, which corresponded to a mean intake of **164 mg/kg/day**. The main findings at the 0.5% level were judged by the study director to be the reversible lower food utilisation efficiency in males, the reversible higher water consumption in females, the reduced plasma total cholesterol which was still present in males during week 18, the reversible serum total protein in males, and the reduced platelets which were still lower than control during week 18. The summary report does not define a no-observed-adverse-effect level (NOAEL).

Ref: 11

Comments of the Applicant on Study Reliability

The in-life portion of this study was conducted in accordance with GLP, which included an audit of the study protocol and study-based inspections at key points during the study. On completion of the study, a protocol amendment was produced to state that only a non-GLP summary report would be produced. Therefore, no claim of GLP compliance can be made for the study report.
On completion of the study, all raw data and records were retained indefinitely. The test material was analysed by GC-MS and by ^{13}C NMR and was considered suitable for test materials with the volatility exhibited by 2-phenoxyethanol. This method was limited to a capability of confirming purity at a level of >95%. The method would be expected to detect other volatile/semi-volatile impurities; none were reported above a level of 0.5%. Given the available information on the purity of the test material, this study is considered less relevant to the safety assessment of 2-phenoxyethanol than the OECD guideline and GLP-compliant study discussed above, which tested a sample of 99.9% purity.

SCCS comment
An amendment to the study plan indicated that only a non-GLP summary report would be produced because the study was "deemed to be a low-priority study for which full reporting will not be necessary". The study documentation is incomplete. Methodology used is not described. Analytical data on the purity of the test substance that might be critical regarding study evaluation is not available. Table 11 of the summary report is missing.
In conclusion, the study is considered of limited value because of incomplete study documentation and unclear circumstances such as the unusual discontinuing of a GLP study and downgrading to a non-GLP status and the late study summary report, i.e. 5 years after the in-life phase.

Study No 3 (1977)

Guideline:	/
Species/strain:	Sprague Dawley rats, CD strain from Charles River
Group size:	15 per sex per dose
Test substance:	2-Phenoxyethanol
Batch:	/
Purity:	Unknown (translucent colourless liquid)
Dose levels:	0, 80, 400, 2000, mg/kg bw/day
Vehicle:	0.5% in gum tragacanth mucilage (5 ml/kg bw)
Route:	Oral, gavage
Exposure period:	90 days
GLP:	No
Report Date:	21 Nov 1977

In a study preceding OECD guidelines and GLP principles, 2-phenoxyethanol was administered via gavage for 13 weeks to CD rats (140-150 g) at doses of 0, 80, 400, and 2000 mg/kg bw/day (Nipa Laboratories, 1977). Five animals, 1 female (mid dose) and 4 males (1 low dose, 2 mid dose, one high dose) died during treatment for unknown reasons and probably not treatment-related. At 2000 mg/kg bw/day, clinical signs including occasional episodes of prostration and lethargy were observed shortly following 2-phenoxyethanol dosing. Females were more affected than males. These episodes occurred as isolated or low-incidence events when dosing was initiated and resolved with continued dosing. Four high-dose females died during the treatment period and their deaths were considered treatment related, although no gross or histopathological changes were identified (one died of bronchopneumonia). Food intake of males at the highest dose was decreased to 94% of controls. The body weights of high-dose male and female rats were decreased by about 17 and 7%, respectively.
Clinical chemistry data showed toxicity to red blood cells and other effects that are associated with this phenomenon (decreased erythrocyte number, decreased packed cell volume and decreased haemoglobin concentration, and kidney inflammation with epithelial cells and polymorphonuclear leukocytes in the urinary sediment) at 2000 mg/kg bw/day.

Liver, kidney and thyroid weights were increased at 2000 mg/kg bw/day. Inflammation of the kidneys was also seen in males at 400 mg/kg bw/day. Minor testicular changes were noted in a few high-dose male rats, but these changes were considered to be of equivocal toxicological significance. The **NOEL** in this study was reported as **80 mg/kg bw/day** based on the finding of inflammation in the kidney in males at 400 mg/kg bw/day.

Ref.: 40

SCCS comment
Deaths of five animals during treatment for unknown reasons and probably not treatment-related suggest poor husbandry or animal treatment in the test facility. The purity of the 2-phenoxyethanol batch used in this study is unknown. The study is considered unreliable.

Study No 4

13-Week study in rats (2003) –MHLW Study, Japan Bioassay Research Center (JBRC)

Guideline:	According to OECD guideline no. 408 (1998)
Species/strain:	Rat/F344/DuCrj
Group size:	10/sex/group
Test substance:	2-Phenoxyethanol
Batch:	WAL4150
Purity:	99.9%
Dose levels:	0, 1250, 2500, 5000, 10000 and 20000 ppm in drinking water; mean intakes calculated to be 96, 185, 369, 687, and 1514 mg/kg/day in males and 163, 313, 652, 1000, and 1702 mg/kg/day in females
Vehicle:	Drinking water
Route:	Oral
Exposure period:	13 weeks
GLP:	Yes
Study Period:	Sept. 10, 2002 – Dec. 12, 2002 (In-life period)
Report Date:	2003

Stability of the test material in drinking water was confirmed by quantitative HPLC analysis (Appendix M 4 of the study report).

Results
Analytical data verified that the test material content in drinking water was within acceptable limits of the target concentrations (101-102%).

In-life findings:
Food consumption was significantly decreased in females at 10,000 ppm (10% relative to controls) and in both sexes at 20,000 ppm (20% relative to controls). Corresponding effects on body weight were observed with significant decreases at 10,000 ppm in females (8% relative to controls) and at 20,000 ppm in both sexes (19% relative to controls). Water consumption was significantly decreased at 5000 ppm in males by approximately 15% during the first 7-8 weeks of dosing and throughout the entire dosing period at 10,000 and 20,000 ppm in females by approximately 30-40% and in males by approximately 15%. Clinical observations included soiled fur in the area of the genitalia in a few animals of both sexes at 5000 and 10,000 ppm and in all animals of both sexes at 20,000 ppm.

Terminal examinations:
Haematological changes were observed including statistically significant reductions in red blood cells (at ≧10,000 ppm in males and females) and haemoglobin (at ≧10,000 ppm in females and at 20,000 ppm in males) and increases in MCV and MCH (at ≧10,000 ppm in males and at 20.000 ppm in females). Reticulocyte count was increased only in females at the top-dose group (20,000 ppm). These changes are consistent with slight anaemia at doses of ≧10,000 ppm. Statistically significant decreases in platelet counts were observed in females at doses ≥10,000 ppm and in males at doses ≥ 5,000 ppm. However, the decrease at 5000 ppm was <10% change from controls and the decreased platelets were not accompanied by evidence of a functional effect since no changes were observed in coagulation parameters (prothrombin and APPT). Furthermore, no effects on platelet counts were seen in the 104-week study in rats (see Section 3.3.7). Therefore, the observation for platelet counts in this 13-week study is considered to be of questionable toxicological significance.

At the end of the treatment period, plasma total protein (at ≥10,000 ppm in males and at 10,000 only in females), glucose (at 20,000 ppm in males), sodium (at 20,000 ppm in males), and calcium (at 20,000 ppm in males) were statistically significantly decreased, whereas A/G ratio (at 20,000 ppm in both sexes), ALP (at 20,000 ppm in females, +32% relative to controls), total cholesterol (at ≥10,000 ppm in males), phospholipid (at ≥10,000 ppm in males), urea nitrogen (at 10,000 ppm in females and at 20,000 ppm in both sexes; both around + 20 to 50% relative to controls), potassium (at ≥10,000 ppm in males) were statistically significantly increased. With the exception of urea nitrogen in both sexes and ALP in females, these statistically significant differences in relation to controls were slight (generally less than 10%) and in several cases were limited to one sex. The ALP elevation was observed only at the highest dose level in only one sex (females) and was not accompanied by other changes in liver enzymes or histopathological findings in liver or bone.

Changes in urinary parameters included a statistically significant decrease in urine pH at ≥ 10,000 ppm in both sexes. This may be attributable to the presence of the acidic metabolite, 2-phenoxyacetic acid at higher concentrations in urine at these doses.

While increases in relative organ weights were observed for several organs (thyroid, lung, brain, testis), most notably in males at 20,000 ppm, these observations were not accompanied by increases in absolute organ weight and were likely related to the decrease in body weight observed at 20,000 ppm. Decreases in absolute organ weights were observed in the thymus, heart, lung, spleen, testis, and ovary at 20,000 ppm and in the adrenal at ≥10,000 ppm. Since there were no histopathological findings pointing to any treatment-related effect in these organs, these findings were considered not to be indicative of organ-related toxicity.

There was a dose-related increase up to 15% in relative liver weight in both sexes at 10,000 and 20,000 ppm, but no increase in absolute liver weight in either sex. Biochemical changes in the serum (increased total cholesterol and phospholipids in males ≥ 10,000 ppm, decreased total protein in males at ≥ 10,000 ppm and in females at 10,000 ppm only, and increased albumin/globulin ratio in both sexes at 20,000 ppm) were slight and did not provide a clear pattern of toxicity, and GOT and GPT were unchanged. Therefore the relative liver weight changes may also be a reflection of the decrease on body weights. No treatment-related histopathological findings were reported in the liver which also supports the conclusion that the liver was not a target organ for toxicity. An increase in relative kidney weight of 10% was seen in females at doses of ≥ 10,000 ppm and in both sexes up to 27% at 20,000 ppm. Absolute kidney weights were not significantly increased in either males or females.

Slight to moderate urothelial hyperplasia of the renal pelvis was observed at 10,000 ppm in males (n=2 of 10 animals) and females (n=1 of 10 animals) and at 20,000 ppm in males (n=6 of 10 animals). Slight to moderate urinary bladder transitional epithelial hyperplasia was observed at 10,000 ppm in females (n=2 of 10 animals) and at 20,000 ppm in females (n=7 of 10 animals). Slight urinary bladder transitional epithelial hyperplasia was observed in one male at 20,000 ppm. As mentioned above, urea nitrogen was increased in both sexes at 20,000 ppm and at 10,000 ppm in females. These observations are all considered treatment-related toxicologically significant effects.

Conclusion

The critical effects in this study pertinent to the establishment of the NOAEL are the effects on red blood cell parameters and the histopathological changes in the kidney and urinary bladder which occurred at doses ≥ 10,000 ppm. The decrease in platelets at 5000 ppm was not considered relevant for the establishment of the NOAEL due to the minimal degree of the effect and the absence of any functional impact on coagulation at any dose. Based on the overall weight of the evidence, the **NOAEL** is considered to be 5000 ppm corresponding

to **369 mg/kg/day** in males and **652 mg/kg/day** in females. The LOAEL is 687 mg/kg/day in males and 1000 mg/kg/day in females.

Subm II, ref. 2

SCCS Comment
The study report does not contain a statement on the version of the testing guideline. However, the report fulfils the formal, methodological and data criteria of the OECD testing guideline 408 (1998).

Study No 5

13-Week study in mice (2003) – MHLW Study, Japan Bioassay Research Center (JBRC)

Guideline:	According to OECD guideline no. 408 (1998)
Species/strain:	Mouse/Cru:BDF1 (B6D2F1)
Group size:	10/sex/group
Test substance:	2-Phenoxyethanol
Batch:	WAL4150
Purity:	99.9%
Dose levels:	0, 1250, 2,500, 5,000, 10,000 and 20,000 ppm in drinking water; mean intakes calculated to be 182, 390, 765, 1178, and 2135 mg/kg/day in males and 236, 478, 948, 1514, and 2483 mg/kg/day in females.
Vehicle:	Drinking water
Route:	Oral
Exposure period:	13 weeks
GLP:	Yes
Study Period:	Sept. 10, 2002 – Dec. 12, 2002 (In-life period)
Report Date:	2003

Results
Analytical data verified that the test material content in diet was within acceptable limits of the target concentrations (101-102%).

In-life findings:
Food consumption was significantly decreased in females at 10,000 ppm beginning at week 7 (8% relative to controls) and in both sexes at 20,000 ppm throughout the entire dosing period (7 and 12% relative to controls in males and females, respectively). Significant decreases in body weight were observed in males at 20,000 ppm throughout the entire dosing period and in females only during week 7 (6% relative to controls). Water consumption was significantly decreased at 2,500 and 5,000 ppm in females by approximately 11% during the last week of dosing and in both sexes throughout the entire dosing period at 10,000 ppm (approximately 25%) and 20,000 ppm (approximately 36%) in both sexes. No treatment-related clinical observations were noted.

Terminal examinations:
Haematological changes were observed including statistically significant reductions in haemoglobin (-5%) and MCHC (-2.5%) and a statistically significant increase in MCV (2%) at 20,000 ppm in females. Haematological changes in males were limited to a statistically significant increase in reticulocytes at 20,000 ppm. The increase in reticulocytes in females at 20,000 ppm was of a similar magnitude as in males (~13%) but was not statistically significant.

In males, at the end of the treatment period, plasma total protein (at 1,250, 5,000, and 20,000 ppm; -4%), total cholesterol (at 5,000 and 20,000 ppm; -11% and -14%),

phospholipid (at ≥5,000 ppm; -10% to -18%), calcium (at 5,000 and 20,000 ppm; around -4%), and phosphorus (at 5,000 and 20,000 ppm; around -18%) were statistically significantly decreased, and ALP (at 20,000 ppm) was statistically significantly increased. Only phospholipids showed a dose-dependent decrease. There were no changes in clinical chemistry parameters in females at any dose.

Changes in urine parameters included a statistically significant decrease in urine pH at ≥ 10,000 ppm in males and at 20,000 ppm in both sexes. This may be attributable to the presence of the acidic metabolite, 2-phenoxyacetic acid at higher concentrations in urine at these doses. Ketone body scores were statistically significantly higher in males and females at 10,000 ppm but not at 20,000 ppm. There was no evidence of a dose-response and this observation was concluded to be an incidental finding.

While increases in relative organ weights were observed for several organs (heart, liver, brain) in males at 20,000 ppm, these observations were not accompanied by increases in absolute organ weights and were likely related to the decrease in body weight observed at 20,000 ppm in males. Absolute thymus weight was decreased in males at 20,000 ppm. Relative kidney weight was increased in both sexes at doses ≥ 10,000 ppm and absolute kidney weight was increased in females at doses ≥ 10,000 ppm (+9% and +12%).

No treatment-related histopathological findings were observed in this study.

Conclusion
Changes in red blood cell parameters in females (haemoglobin, MCHC, and MCV) and males (reticulocytes) at 20,000 ppm suggest a slight haemolytic anaemia at the high dose but no effect at lower doses. Changes in clinical chemistry parameters that may suggest a treatment-related effect on the liver including decreases in cholesterol and phospholipid at doses of ≥ 5,000 ppm in males, although there was no evidence of any histopathology in the liver and no increase in liver enzymes (GPT, GOT). Relative increases of kidney weight occurred in both sexes at higher dose levels (≥ 10000 ppm). While it is possible that the liver-related changes in cholesterol and phospholipid are not adverse, these findings in males were very conservatively used as the basis for establishing the **NOAEL** in this study at 2500 ppm, corresponding to an intake of **390 mg/kg/day**.

Subm II, ref. 3

3.3.5.4 Sub-chronic (90 days) toxicity (dermal)

Guideline:	Equivalent to OECD guideline no. 411 (1981) (see SCCS comment)
Species/strain:	Rabbit/New Zealand White
Group size:	10 per sex per dose
Test substance:	Ethylene glycol phenyl ether (2-Phenoxyethanol)
Batch:	53 (C44172)
Purity:	99.9%
Dose levels:	0, 50, 150, 500 mg/kg bw/day
Vehicle:	Undiluted
Route:	Dermal, occlusive conditions
Exposure period:	6 hours per day, 5 days/week for 13 consecutive weeks
GLP:	Yes
Study Period:	23 Oct 1985 - 08 Oct 1986
Report Date:	1986

An area (approximately 10 x 15 cm) on the back of each animal was clipped free of hair prior to initiation of dosing and periodically as needed during the course of the study. The test material was uniformly spread over the clipped area. An occlusive bandage of absorbent gauze and non-absorbent cotton was placed on the back over the dosing area and held in place using a Lycra/spandex jacket. The bandage and jacket were removed

approximately 6 hours after each dose was applied. Dosing volume was approximately 0.05 to 0.50 ml/kg bw/day and was adjusted weekly based on body weight. Control rabbits received 0.5 ml/kg bw/day of distilled water.

The animals were examined daily for general state of health and clinical symptoms of toxicity. The animals were weighed prior to the first exposure and weekly thereafter. The condition of the skin at the application site was assessed prior to each daily dose for the first two weeks and approximately weekly thereafter using a modified Draize scoring system. Blood samples for haematology and clinical chemistry were collected 1 week prior to exposure, at week 4 of dosing, and immediately prior to necropsy. Haematological evaluation included: red blood cell (RBC), white blood cell (WBC), and platelet (PLT) counts, haemoglobin concentration, packed cell volume and RBC indices (MCV, MCH, MCHC). Reticulocyte counts were also performed for all animals in the control and high-dose groups at 13 weeks. Clinical chemistry parameters evaluated included: alanine aminotransferase activity (ALAT), aspartate aminotransferase activity (ASAT), urea nitrogen, alkaline phosphatase activity, glucose, total protein, albumin, globulin and total bilirubin.

A complete set of organs was collected from each animal and was preserved in neutral phosphate-buffered 10% formalin. The brain, heart, liver, kidneys, and testes were weighed and relative organ weights were calculated. The lungs were infused with formalin to their approximate normal inspiratory volume, while the nasal cavity was flushed with formalin via the pharyngeal duct to ensure rapid fixation of the tissues. A complete histopathological examination of tissues was conducted on animals in the control and high-dose group.

Results
There were no mortalities at any dose. The mean body weights of male and female rabbits were not affected by treatment with 2-phenoxyethanol during the course of the study. There were no treatment-related effects in either males or females on clinical chemistry or haematology parameters measured at either 4 or 13 weeks. A statistically significant decrease in ASAT activity was reported for the females of the 150 mg/kg bw/day dose group after 4 weeks of treatment and males in the group receiving 50 mg/kg bw/day showed an increased ALAT activity at study termination. However, these effects did not show a dose-response relationship, nor were they seen at the other sampling time points. Therefore, these effects were not considered to be treatment-related. No treatment-related effects were observed on organ weights or relative organ weights. Dermal irritation scores at the site of application revealed sporadic findings of erythema and very slight scaling in male and female rabbits of the 500 mg/kg bw/day dose group. These changes were not associated with gross or histopathological changes and were not considered toxicologically significant. There were no treatment-related gross pathologic or histopathological changes observed in either males or females at 500 mg/kg bw/day.

Conclusion
Based on the lack of treatment-related effects on body weight, organ weights, haematological and clinical chemistries and gross and histopathological examinations, the no-observed-adverse-effect level (**NOAEL**) for systemic toxicity was concluded to be **500 mg/kg bw/day** under the conditions of this study.

Ref.: 12, 13

SCCS Comment
The study report does not contain a statement on the testing guideline. However, the report fulfils the formal, methodological and data criteria of the OECD testing guideline 411 (1981).
The SCCS considers this study as a key study for the safety assessment of 2-phenoxyethanol because the dermal route is the relevant route for 2-phenoxyethanol as a cosmetic ingredient and the rabbit has been shown to be the most sensitive species to haematotoxic effects when systemically exposed to 2-phenoxyethanol.

To account for the dosing schedule used in this study, the NOAEL should be multiplied by a factor of 5/7 to give an **adjusted NOAEL** of **357 mg/kg bw/day**.

Table 3

90-DAY DERMAL EXPOSURE IN RABBITS: HEMATOLOGY AFTER 13-WEEKS OF EXPOSURE[a]

									Differential WBC and RBC morphology						
Dose (mg/kg/day)	RBC ×10⁶ /MM³	HGB G/DL	PCV %	MCV (μ³)	MCH (g × 10⁻¹²)	MCHC %	PLT ×10³ /MM³	WBC ×10³ /MM³	SEG %	LYM %	MONO %	EOS %	BASO %	RBC MORPH	RETICS %
							MALES								
0	6.73	14.3	49.2	73	21.2	29.0	348	6.7	34	62	1	0	2	N, N[b]	1.6
	0.34	0.9	2.5	2	0.6	0.4	72	1.4	6	6	1	1	1	0	0.3
	N = 10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
500	6.55	13.8	48.2	74	21.1	28.7	330	7.0	33	63	2	0	3	N, N	1.3
	0.39	0.8	2.5	2	0.8	0.6	43	1.1	8	9	2	1	1	0	0.4
	N = 10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
							FEMALES								
0	6.03	12.8	44.0	73	21.2	29.0	315	6.5	36	60	1	0	2	N, N	1.5
	0.46	0.6	2.5	2	1.0	0.6	50	1.5	8	9	1	0	1	0	0.2
	N = 10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
500	6.08	12.8	44.7	74	21.1	28.6	324	6.5	35	62	1	0	2	N, N	1.5
	0.28	0.6	1.6	2	0.9	0.8	66	1.4	10	11	1	0	1	0	0.3
	N = 10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

[a] Data are mean and SD for the specified number of animals (*N*). There were no statistically identified differences from control mean, $\alpha = 0.05$. RBC indices (MCV, MCH, MCHC) and differential WBC data were not statistically compared.

[b] N, N indicates normocytic, normochromic.

3.3.5.5 Chronic (> 12 months) toxicity

See 3.3.7.

3.3.6 Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation test

Guideline:	OECD 471 (1997)
Species/strain:	*S. typhimurium*, TA98, TA100, TA1535, TA1537, and *E. coli* WP2 uvr A
Replicates:	Triplicates were investigated per test concentration
Treatment:	Plate incorporation and pre-incubation test without and with S9 mix from rat livers (Aroclor 1254-induced)
Test substance:	Protectol® PE (2-Phenoxyethanol)
Batch No.:	66-4287
Purity:	99.9%
Concentrations:	0, 20, 100, 500, 2500, and 5000 µg/plate with and without S9 mix
Stability:	Stability in DMSO over 4 hrs and in water over 96 hrs was verified by re-analysis
Vehicle:	DMSO
GLP:	Yes
Study Period:	19 September 2001 – 5 December 2001

2-Phenoxyethanol was assessed for its potential to induce gene mutations according to the plate incorporation test and the pre-incubation test using *S. typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537, and in *E. coli* WP2 uvr A. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system.
The test was performed with and without liver microsomal (S9-mix) activation. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations: 20, 100, 500, 2500, and 5000 µg/plate.

Results
In the plate incorporation test, the plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used. No toxic effects, evident either as a reduction in the number of revertants or as a reduction in the bacterial background lawn, occurred in the test groups with or without metabolic activation. In the pre-incubation test, a slight decrease in the number of revertants and/or slight reduction in the titer was occasionally observed depending on the strain and test conditions.
No biologically relevant increase in revertant colony numbers of any of the five tester strains was observed following treatment with 2-phenoxyethanol at any concentration level, either in the presence or in the absence of metabolic activation (S9-mix). There was also no evidence of any increase in mutation rates with increasing concentrations. Appropriate reference mutagens were used as positive controls and showed a distinct increase in revertant colonies.

Conclusion
Under the experimental conditions used in this gene mutation test, 2-phenoxyethanol was considered not genotoxic (mutagenic) in bacteria.

Ref.: 19

Gene mutation test in Chinese Hamster V79 cells

Guideline:	OECD 476 (1997)
Species/strain:	Chinese Hamster Lung Fibroblasts (V79)
Replicates:	duplicate cultures in two independent experiments
Test substance:	Protectol® PE (2-Phenoxyethanol)
Batch No.:	664287
Purity:	99.9%
Concentrations:	87.5, 175, 350, 700, and 1400 µg/ml with and without S9-mix (Experiments I and II)
Treatment:	4 h treatment with and without S9-mix; expression period 72 h and selection period of 10-15 days (experiment I and II)
Vehicle:	DMSO
GLP:	Yes
Study Period:	17 September 2001 – 26 February 2002

The test item 2-phenoxyethanol was assessed for its potential to induce gene mutations at the *Hprt* locus using V79 Chinese hamster cells. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-test on toxicity. The study was performed in two independent experiments. The cells were treated for 4 hours followed by an expression period of 72 h to fix the DNA damage into a stable *Hprt* mutation.
Negative (culture medium) and solvent controls (DMSO) were included in both experiments I and II. Ethyl methane sulfonate (EMS) at a final concentration of 150 µg/ml was used as a positive control in experiments I and II without metabolic activation (S9 mix), and 7,12-dimethylbenz(a)anthracene (DMBA) at a final concentration of 2.7 µg/ml was used in experiments I and II with metabolic activation (S9-mix).

Results
The test was considered valid since the numbers of mutant colonies per 10^6 cells found in the negative and solvent controls fell within the laboratory's historical control data, the positive control substances produced a significant increase in mutant colony frequencies, and the cloning efficiency of the negative and solvent controls exceed 50%. No precipitation of the test item was observed in any of the main experiments and no cytotoxicity was observed up to the maximal concentration of 1400 µg/mL (approximately 10 mM).
No biologically relevant concentration dependent increase in mutant frequency was observed up to the maximal concentration. An increase of the induction factor exceeding the threshold of three times the corresponding solvent control occurred in the second culture of experiment I without metabolic activation at 350 µg/mL and in the second culture of experiment I at 1400 µg/mL in the presence of metabolic activation. However, this increase was judged to be based upon the rather low solvent controls. Compared to the corresponding negative control, the threshold was not reached. The historical range of negative and solvent controls was only exceeded in the first culture of the second experiment at 87.5 and 175 µg/mL with metabolic activation. Although this increase exceeded the historical range of solvent controls, it was not concentration-related and no comparable effect occurred in the parallel culture under identical conditions or in the first experiment. Therefore, all these isolated positive effects were judged as not biologically relevant.

Conclusion
Under the experimental conditions used, 2-phenoxyethanol was considered not mutagenic in this *Hprt* gene mutation test in V79 cells.

Ref.: 20

SCCS comment
No relevant toxic effect was observed up to the maximum concentration (approximately 10 mM) in the presence and absence of metabolic activation. Therefore this gene mutation test in mammalian cells is of limited value.

Gene mutation test in Chinese Hamster Ovary (CHO) cells

Guideline:	OECD 476 (1997)
Species/strain:	Chinese Hamster Ovary (CHO-K_1-BH_4) Cells
Replicates:	duplicate cultures in two independent experiments
Test substance:	2-Phenoxyethanol (Phenoxetol)
Batch No.:	53
Purity:	99.83%
Concentrations:	2500, 3000, 3250, 3375 and 3500 µg/ml without S9-mix 2000, 2500, 3000, 3250 and 3500 µg/ml with S9-mix
Vehicle:	Culture medium
Treatment:	4 h with and without S9-mix; expression period 7 days, selection growth 7-9 days.
GLP:	Yes
Study Period:	06 March 1987 – 21 August 1987

2-phenoxyethanol was assessed for its potential to induce gene mutations at the H*prt* locus using Chinese hamster ovary (CHO) cells. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a preliminary toxicity assay measuring relative cell survival. In the main test, cells were treated for 4 h, followed by an expression period of 7 days to fix the DNA damage into a stable *Hprt* mutation. In the main test, toxicity was also measured as percentage cell survival of the treated cultures relative to the cell survival of the solvent control cultures.
Ethyl methane sulfonate (EMS) at a final concentration of 621 µg/ml was used as a positive control without metabolic activation, and 2-methylcholanthrene (2-MCA) at a final

concentration of 4.03 µg/ml was used as a positive control with metabolic activation (S9 mix).

Results
In the preliminary toxicity test, a concentration of 3500 µg/ml was associated with relative cell survival rates of 6.2% and 3.4% in the non-activation and activation tests, respectively. A concentration of 2500 µg/ml was non-toxic to the cells. In the mutation test conducted in the absence of S-9 mix, relative cell survival was 25.6% at 3500 µg/ml. In the mutation test conducted with S-9 mix, relative cell survival was 3.8% at 3500 µg/ml, which was concluded to be excessive. The relative cell survival at 3250 µg/ml in the presence of S-9 mix was 14.2%.

A biological increase in the mutant frequency after treatment with 2-phenoxyethanol was not observed at any concentration tested, both with and without metabolic activation.
The positive controls EMS and 2-MCA caused statistically significant increases in the frequencies of mutants, indicating that the test was valid.

Conclusion
Under the experimental conditions reported, 2-phenoxyethanol did not induce gene mutations at the *Hprt* locus in CHO cells and, consequently, 2-phenoxyethanol is considered to be non-mutagenic in this test.

Ref.: 21

***In vitro* chromosome aberration test in Chinese hamster V79 cells**

Guideline:	OECD 473 (2004; draft guideline)
Species/strain:	Chinese Hamster Lung Fibroblasts (V79)
Replicates:	duplicate cultures in two independent experiments
Test substance:	Protectol® PE (2-phenoxyethanol)
Batch No.:	664287
Purity:	99.9%
Vehicle:	De-ionised water
Concentrations: with and without S9-mix	Experiment I: 43.8, 87.5, 175, 350, 700, and 1400 µg/ml Experiment II: 175, 350, 525, 700, 1050 and 1400 µg/mL without S9-mix 175, 350, 700, and 1400 µg/mL with S9-mix
Treatment:	Experiment I: 4-h treatment with and without S9-mix; harvest time 18 h after the start of treatment
Experiment II:	18-h treatment without S9-mix; harvest time 18 h or 28 h after the start of treatment 4-h treatment with S9-mix; harvest time 28 h after the start of treatment
GLP:	Yes
Study period:	17 September 2001 – 21 February 2002

Protectol® PE has been investigated for the induction of chromosomal aberrations in V79 cells both in the absence and presence of metabolic activation. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results from a pre-test on cell growth inhibition after 4 h and 24 h treatment. The cytotoxicity of the test substance was examined using the determination of the cell number. The general experimental conditions in this pre-test were the same as described below for the cytogenetic main experiment.
In experiment I, the exposure period was 4 hours with and without metabolic activation. In experiment II, the exposure period was 4 hours with S9-mix and 18 hours without S9-mix. The cells were harvested 18 h (experiment I and II) and 28 h (experiment II) after start of treatment with the test substance. In the main tests, toxicity was measured by cell counts

and by the determination of the mitotic index. Ethyl methane sulfonate (EMS) at a concentration of 200 µg/mL was used as a positive control in the absence of S9-mix and cyclophosphamide (CPA) at a concentration of 0.7 µg/mL was used as a positive control in the presence of S9-mix.

Results
No cytotoxicity was observed up to the maximum 2-phenoxyethanol concentration of 1400 µg/mL (approximately 10 mM). The positive controls showed clear increases in the cells with structural chromosome aberrations.
In both experiments, a biologically relevant increase in cells with chromosome aberrations was not found independent of the presence or absence of S9-mix and of treatment times.
A single statistically significant increase ($p<0.05$) in aberrant cells was observed in experiment I after 4 hours treatment at 2-phenoxyethanol at 1400 µg/mL without S9-mix. Although this increase in aberrant cells (4% aberrant cells, exclusive gaps) was statistically significant compared to the low in the corresponding solvent control (1% aberrant cells, exclusive gaps), the value is within the historical control data range. Therefore, this finding is regarded as being biologically irrelevant.

Conclusion
Under the experimental conditions of this study, Protectol® PE did not induce structural chromosome aberrations in V79 cells, and consequently, Protectol® PE is considered to be non-clastogenic in this test.

Ref.: 22

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

Mouse bone marrow micronucleus test

Guideline:	OECD 474 (1997)
Species/strain:	Mouse, strain NMRI
Group size:	6 males per dose group
Test substance:	Protectol® PE (2-Phenoxyethanol)
Batch No.:	664287
Purity:	99.9%,
Dose levels:	125, 250, and 500 mg/kg bw (24 h) and 500 mg/kg bw (48 h)
Route:	single intraperitoneal applications
Vehicle:	0.5% carboxymethylcellulose
Sacrifice times:	24 (all doses) and 48 hours (high-dose only) after start of treatment
GLP:	Yes
Study Period:	02 October 2001 – 12 February 2002

2-Phenoxyethanol was investigated for induction of micronuclei in the bone marrow cells of male NMRI mice. Dose selection was based on the result of a pre-experiment, performed under identical conditions as in the mutagenicity study, for toxicity in which 2 to 5 mice/sex were treated with doses up to 2000 mg/kg bw. The animals were examined for acute toxic symptoms at intervals of around 1, 2-4, 6, 24, 30 and 48 h after administration.
In the main experiment, mice were exposed orally to 0, 25, 50 and 100 mg/kg bw. The animals of the highest-dose group were examined for acute toxic symptoms at intervals around 1, 2-4, 6 and 24 h after treatment. Bone-marrow cells were collected 24 h or 48 h (high dose only) after dosing. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/TE ratio). Bone marrow preparations were stained and examined microscopically for the NCE/TE ratio and micronuclei. Negative and positive controls were in accordance with the OECD guideline. 2-Phenoxyethanol was dissolved in 0.5% carboxymethylcellulose and was administered at doses of 125, 250, and 500 mg/kg bw (24 hours) and 500 mg/kg bw (48 hours) to groups of six male NMRI mice. For the high dose, two groups were treated to allow sampling after 24 and 48 hours.

Mice were sacrificed 24 hr after dosing for all dose groups and also in an additional high dose group at 48 hr after dosing. Femoral bone marrow was collected and slides were prepared from the bone marrow preparations, stained with May-Grünwald/Giemsa and evaluated (coded) for the number of polychromatic erythrocytes (PCE) with micronuclei. At least 2000 PCEs per animal were analysed. In addition, the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes.

Results
Signs of toxicity (reduction of spontaneous activity, eyelid closure, apathy, abdominal position, Straub position) were observed in animals dosed with 500 mg/kg bw. The mean number of normochromatic erythrocytes (NCEs) was increased after treatment at 250 and 500 mg/kg bw as compared to the mean value of NCEs of the vehicle control, indicating that 2-phenoxyethanol showed evidence of cytotoxicity in the bone marrow.
No biologically relevant increase in the number of cells with micronuclei in the mice of any of the 2-phenoxyethanol-treated groups was observed compared to the respective vehicle control groups. The mean values of micronuclei observed after treatment with 2-phenoxyethanol were not statistically significantly different from the controls. The positive control (cyclophosphamide) produced a marked induction of micronuclei, and both the positive and the vehicle control were well within the range of historical control data of the performing laboratory, thus demonstrating the validity and sensitivity of the used test system.

Conclusion
Under the experimental conditions used, 2-phenoxyethanol did not induce an increase in the number of cells with micronuclei in erythrocytes of treated mice and, consequently, 2-phenoxyethanol was not genotoxic (clastogenic and/or aneugenic) in erythrocytes of mice in this test.

Ref.: 23

Rat bone marrow chromosome aberration test

Guideline:	OECD 475
Species/strain:	Rat/Sprague Dawley
Group size:	5 animals per sex per dose group
Test substance:	2-Phenoxyethanol, PHENOXETOL
Batch No.:	53
Purity:	99.83%
Dose levels:	0, 280, 933, and 2800 mg/kg bw
Duration of Exposure:	6, 24, and 48 h after start of treatment
Route:	oral, gavage single doses
Vehicle:	Corn oil
GLP:	In compliance
Study Period:	27 May 1987 – 04 Feb 1988

2-Phenoxyethanol was investigated for induction of chromosome aberrations in the bone marrow cells of rats. The high dose was chosen based on a pre-experiment for toxicity in which the LD50 value (moving average method) was reported as 2937 and 4013 mg/kg bw for males and females, respectively. In the main experiment, 2-Phenoxyethanol was administered in corn oil vehicle at doses of 280, 933, and 2800 mg/kg bw to groups of five male and five female Sprague Dawley rats by oral gavage. Single doses were administered to the animals at a total volume of 10 ml/kg bw. Vehicle control groups received corn oil and concurrent positive control groups received 2000 mg/kg bw trimethylphosphate by oral gavage.
Approximately 3 hours before sacrifice the animals were injected with 2 mg/kg colchicine. The bone marrow was removed by aspiration and slides were prepared and stained with Giemsa. To determine cytotoxicity of 2-phenoxyethanol and thus exposure of the target

cells, mitotic indices were determined as the number of cells in metaphase among 1000 cells for each rat. Based on the absence of an increase in the incidence of aberrations in the mid- and high-dose groups, the low-dose group was not scored.

Results
Cytogenetic data from one male animal in the vehicle-control group and one male in the high-dose group could not be collected at 6 h due to technical errors. One male in the high-dose group died before the scheduled euthanasia time of 6 h. For the 24-h exposure groups, one male in the vehicle-control group and two males in the high-dose group died before the scheduled sacrifice time. For the 48-h exposure groups, one vehicle-control male and three high-dose males did not survive to the scheduled sacrifice time. Among females treated with 2800 mg/kg bw, 1, 3, and 3 animals died before the scheduled euthanasia times of 6, 24 and 48 h, respectively. All positive controls and animals treated with lower doses of test material survived.
Data on mitotic indices indicated that there was no excessive cell toxicity among groups treated with 933 and 2800 mg/kg bw; occasionally a decreased mitotic index was observed. There were no biologically relevant and statistically significant increases in the number of cells with chromosome aberrations in groups treated with 933 or 2800 mg/kg bw test material at any time point compared to vehicle controls. The positive controls induced a significant increase in the total number of aberrations (48 and 54 in males and females, respectively).

Conclusion
Under the experimental conditions used, 2-phenoxyethanol did not induce an increase in the number of cells with chromosomal aberrations in erythrocytes of treated rats and, consequently, 2-phenoxyethanol was not genotoxic (clastogenic) in erythrocytes of rats in this test.

Ref.: 24

SCCS comment
As no clear effect on the mitotic indices was observed, it is not clear whether the target cells were sufficiently exposed. Consequently, the test is of limited value.

Unscheduled DNA Synthesis

Guideline:	OECD 486
Species/strain:	Rat/Wistar
Group size:	4 males per dose group
Test substance:	Protectol® PE (2-Phenoxyethanol)
Batch No.:	664287
Purity:	99.9%
Dose levels:	0, 875 and 1750 mg/kg
Preparation Time:	2 and 16 hrs post dosing
Route:	oral, gavage single doses
Vehicle:	0.5% carboxymethylcellulose
GLP:	Yes
Study Period:	14 September 2001 – 12 February 2002

The test doses were based on the results of a dose-range finding pre-experiment in which animals (males and females) were dosed with 1500, 1750, and 2000 mg kg bw and monitored for clinical signs of toxicity at 1-6, 24 and 48 hr after dosing. Based on the results, 1750 mg/kg bw was estimated to be suitable as maximum tolerable dose for the main experiment. The main experiment was conducted using only male rats since no sex differences in toxicity were observed.
In the main experiment, 2-phenoxyethanol was administered to four male rats per dose per exposure time at doses of 875 and 1750 mg/kg bw. Animals were sacrificed at either 2 hrs or 16 hrs following dosing. Two additional vehicle control groups of four rats each received a single oral gavage dose of vehicle. Positive control groups were dosed with 40 mg/kg bw

N,N'-dimethylhydrazine dihydrochloride at 2 hrs prior to sacrifice or with 100 mg/kg bw 2-acetylaminofluorene at 16 hrs prior to sacrifice.
Hepatocytes for UDS analysis were collected approximately 2 h and 16 h after administration of 2-phenoxyethanol using 0.05 % (W/v) collagenase.
At least 90 minutes after plating, the cells were incubated for 4 h with 5 µCi/ml ^{3}H-thymidine (specific activity 20 Ci/mmol) followed by overnight incubation with unlabelled thymidine. Evaluation of autoradiography was done after 14 days. UDS was reported as net grain counts: the nuclear grain count subtracted with the average number of grains in a heavily labelled nuclear sized area adjacent to the nucleus. Increased net grains should be based on enhanced nuclear grain counts rather than on decreased cytoplasmic grain counts. Unscheduled synthesis was determined in 50 randomly selected hepatocytes on 2 replicate slides per rat from at least 3 treated rats.

Results
The following symptoms were reported in the dose rage-finding pre-experiment: reduction of spontaneous activity, change in abdominal position, eyelid closure, apathy, ruffled fur and death. The symptoms regarding abdominal position and apathy were no longer noted after 48 hr.

Additional animals were used due to high mortality rates reported at the 16 hr preparation interval at the test dose of 1750 mg/kg bw. In total, 5 out of the 10 animals treated at this dose died. The viability of the hepatocytes was not substantially affected due to the *in vivo* treatment with the test substance. The inter-individual variations obtained for the total number and the viability of the isolated hepatocytes were in the range of the historical laboratory control. None of the groups dosed with 2-phenoxyethanol showed an increase in net nuclear grain counts. Treatment with the positive control substances produced clear increases in the number of net nuclear grain counts.

Conclusion
Under the experimental conditions reported, 2-phenoxyethanol did not induce DNA damage leading to unscheduled DNA synthesis and, consequently, 2-phenoxyethanol is not genotoxic in rats in this *in vivo* UDS test.

Ref.: 25

Summary of the Applicant on mutagenicity/genotoxicity
2-Phenoxyethanol was tested for mutagenic/genotoxic potential in an adequate battery of *in vitro* and *in vivo* tests with various endpoints. 2-Phenoxyethanol was not mutagenic in the Ames test at concentrations up to 5000 µg/plate with and without metabolic activation. Further tests for gene mutation at the *Hprt* locus in mammalian cells also showed no evidence of mutagenic potential either with or without metabolic activation. *In vitro* chromosome aberration tests showed no evidence of a clastogenic effect.
In vivo tests also showed no mutagenic/genotoxic potential for 2-phenoxyethanol. *In vivo* micronucleus and chromosome aberration tests conducted in mice and rats showed no evidence of clastogenic potential. Testing for DNA damage in an UDS test in rats also showed no evidence of genotoxicity.
Consequently, based on the present reports, 2-phenoxyethanol can be considered to have no *in vivo* genotoxic potential and to be of no genotoxic hazard to humans. Additional tests are unnecessary.

3.3.7 Carcinogenicity

1) Carcinogenicity study in rats
MHLW Study, Japan Bioassay Research Center (JBRC), Submission II

Guideline: According to OECD guideline no. 451 (1981)
Species/strain: Rat/F344/DuCrlCrlj

Group size:	50/sex/group
Test substance:	2-Phenoxyethanol
Batch:	PKF5373
Purity:	99.8-99.9%
Dose levels:	0, 2500, 5000, and 10000 ppm in drinking water (for dose calculations based on mg/kg bw/day see below)
Vehicle:	Drinking water
Route:	Oral, via drinking water
Exposure period:	104 weeks
GLP:	Yes
Report Date:	June 2007

The following summary of this study is based on the official English translation of the study summaries available on the MHLW website along with the data tables also available on the website (Additional *Ref. 3*).

In this chronic oral toxicity study, 2-phenoxyethanol was administered daily for 104 weeks to rats of both sexes via the drinking water at concentrations of 2500, 5000, and 10000 ppm. These drinking water concentrations were listed in the study report (Section III-6) to be equivalent to 141, 277, and 551 mg/kg/day in males and 205, 406, and 811 mg/kg/day in females. Using the chemical intake data from Appendices F1 and F2 in the study report, the mean intakes of the 2-phenoxyethanol across the duration of the study for each of the three dose levels were estimated to be 124, 249, and 510 mg/kg/day in males and 191, 380, and 795 mg/kg/day in females. High-dose selection for this study was based on the previously completed 13-week oral toxicity study in rats described above.

Results
In-life findings:
There was no significant difference in survival rate between groups of animals treated with 2-phenoxyethanol and the controls. Growth rates in males at 10,000 ppm and in females at all doses were suppressed relative to controls. Terminal body weights for the 2500, 5000, and 10,000 ppm groups were 98%, 98%, and 94% in males and 95%, 96%, and 89% in females of the values for the respective controls and statistically significant only in the high-dose groups. Food consumption was decreased by 2% in females at 2500 ppm and 5000 ppm and by 6% in both sexes at 10,000 ppm. Water consumption was decreased in the 5000 and 10,000 ppm dose groups in males and in all of the dose groups in females during the first half of the two-year dosing period when compared to the respective controls.
Clinical observations included soiled fur around the genitalia in males at 10,000 ppm and in females at all doses.

Terminal examinations:
A statistically significant increase in mean corpuscular volume (MCV) by about 5% relative to controls was observed in females at 10,000 ppm. A statistically significant decrease in mean corpuscular haemoglobin (MCH) was observed in males at 2500 ppm but MCH values at 5000 and 10,000 ppm were not significantly different from controls. Based on the absence of changes in other haematological parameters in both sexes and the lack of a dose response associated with the change in MCH in males, these observations were not considered indicative of a toxicologically significant haematological effect.

Changes in clinical chemistry parameters included statistically significant increases in ALT and AST by about 50% at 10,000 ppm in males and in total bilirubin (+24%) and urea nitrogen (+8%) at 10,000 ppm in females as well as a statistically significant decrease by 44% in triglycerides at 10,000 ppm in females (all changes relative to controls). Statistically significant decreases of marginal magnitude were observed for total protein and creatinine at 10,000 ppm in males and a statistically significant increase of marginal magnitude was observed for urea nitrogen at 10,000 ppm in females. These were not considered to be toxicologically significant treatment-related changes.

Changes in urine parameters included a statistically significant decrease in urine pH at 10,000 ppm in females. The study report summary states that this might be due to urinary excretion of 2-phenoxyacetic acid, the major metabolite of 2-phenoxyethanol. A decrease in urinary protein and in the incidence of ketone bodies in urine was observed in females at 10,000 ppm. These changes were considered to have no toxicological significance.

Increases in relative organ weights for heart (females) and brain (by 6% in males and 9% in females, respectively) were observed at 10,000 ppm. Relative ovary weight was increased by 15, 34 and 34% in the low-, mid- and high-dose group, respectively. However, no changes in absolute organ weights were observed for these organs and no histopathological changes were observed. Therefore these relative organ weight changes were considered to be of no toxicological significance. Increases in absolute and relative adrenal weight were observed in males at all doses by 22-39%, while in females a decrease in absolute adrenal weight by 11% was seen at 10,000 ppm. Given the variability in the measurements, and the high background incidence of pheochromocytoma in the male F344/DuCrlCrlj rat, the increase in adrenal weights may be confounded and is therefore not considered toxicologically relevant. No changes were observed for absolute or relative liver weights. Absolute kidney weight was increased in females and relative kidney weights were increased in both sexes at 10,000 ppm. In the absence of histopathological findings in the kidney in females, the kidney weight change was considered to not be of toxicological significance. Histopathological findings in the kidney in males are discussed below.

There was no evidence of a treatment-related increase in neoplastic lesions in either sex in this study. Non-neoplastic histopathologic findings included an increase in the incidence and severity of renal pelvis urothelial hyperplasia and an increase in the incidence of renal papillary mineralisation and necrosis in males at 10,000 ppm. These changes in males were minimal (grade 1 or 2). No notable histopathological changes were observed in females. From these results, it was concluded that the kidney was a target organ for toxicity in males but not in females in this study.

Conclusions
There was no evidence of carcinogenic activity of 2-phenoxyethanol in male or female rats. There were no clear treatment-related haematological effects in this study. The kidney was a target organ in males in this study with an increased incidence of slight to moderate urothelial hyperplasia and slight papillary mineralisation and necrosis observed in males at 10,000 ppm. No histopathological findings in the kidney were observed in females. While liver enzymes (AST, ALT) were increased in males at 10,000 ppm and increased bilirubin and decreased triglycerides were observed in females at 10,000 ppm, histopathology of the liver was unremarkable in both sexes. Based on the histopathological findings in the kidney in males, the **NOAEL** is established as 5000 ppm corresponding to **249 mg/kg/day**.

Subm II, ref. 4

2) Carcinogenicity study in mice
MHLW Study, Japan Bioassay Research Center (JBRC), Submission II

Guideline:	According to OECD guideline no. 451 (1981)
Species/strain:	Mouse/B6D2F1/Crlj
Group size:	50/sex/group
Test substance:	2-Phenoxyethanol
Batch:	PKM4201, PKF5373
Purity:	≥ 99.8%
Dose levels:	0, 5000, 10000 and 20000 ppm in drinking water (for dose calculations based on mg/kg bw/day, see below)
Vehicle:	Drinking water
Route:	Oral, via drinking water

Exposure period: 104 weeks
GLP: Yes
Report Date: 2007

The following summary of this study is based on the official English translation of the study summaries available on the MHLW website along with the data tables, also available on the website (Ref. 4).

In this chronic oral toxicity study, 2-phenoxyethanol was administered daily for 104 weeks to mice of both sexes via the drinking water at concentrations of 5000, 10,000 and 20000 ppm. These drinking water concentrations were reported in the study report (Section III-6) to be equivalent to 543, 1011, and 1815 mg/kg/day in males and 650 1166, and 2144 mg/kg/day in females. Using the chemical intake data from Appendices F1 and F2 in the study report, the mean intakes of the 2-phenoxyethanol across the duration of the study for each of the doses levels were estimated to be 468, 898, and 1701 mg/kg/day in males and 586, 1072, and 2058 mg/kg/day in females. High dose selection for this study was based on the previously completed 13-week oral toxicity study in mice described above.

Body weight, food consumption and drinking water consumption were measured weekly for the first 13 weeks and then every 4 weeks through week 77, at week 78, every 4 weeks through week 102 and at week 104.

The following observations/examinations were included in the study: clinical signs and mortality, urinalysis, haematology and blood chemistry, gross pathology at necropsy, organ weights (adrenal, testis, ovary, heart, lung, kidney, spleen, liver, and brain) and histopathology (both non-neoplastic and neoplastic lesions).

At the end of treatment, blood samples were drawn for haematology and plasma chemistry analyses. Haematological evaluation included: red blood cell count (RBC), haemoglobin (Hgb), haematocrit (Hct) mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, reticulocytes, white blood cell count (WBC), and differential WBC count.
Plasma chemistry analyses included total protein (TP), albumin (Alb), A/G ratio, total bilirubin, glucose, total cholesterol, triglyceride, phospholipid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), γ-glutamyl transpeptidase, (γ-GTP), creatine kinase (CK), urea nitrogen, creatinine, sodium, potassium, chloride, calcium, and inorganic phosphorus.

Urine samples were collected for urinalyses at the end of treatment. Parameters evaluated included pH, protein, glucose, ketone body, bilirubin, occult blood, and urobilinogen.

Results
In-life findings:
There was no significant difference in survival rate between treated and control animals. Growth rates in males and females administered 10,000 ppm and above were suppressed in a dose-related manner. Terminal body weights for the 5000, 10,000, and 20,000 ppm dose groups were 98%, 84%, and 72% for males and 100%, 94%, and 79% for females, as compared to the respective controls. Food consumption was decreased in both males and females administered 10,000 ppm and above. A dose-dependent decrease in water consumption relative to controls was noted in all treatment groups of both sexes.
No clinical signs were observed in any of the treatment groups in either sex.

Terminal examinations:
A statistically significant decrease in white blood cell count by 26% was observed in males at 20,000 ppm. A statistically significant increase in haematocrit by 10% was observed in females at 20,000 ppm. These changes were not considered to be treatment-related. No other changes in haematology were observed.

Statistically significant decreases in ALT, AST, and LDH were observed in females at 20,000 ppm and a decrease in ALT was observed in males at 10,000 ppm and 20,000 ppm. These decreases in clinical chemistry indicators were not considered to be toxicologically relevant. A marginal but statistically significant increase in alkaline phosphatase (ALP) was observed in females at 20,000 ppm. Decreases in cholesterol (at 10,000 and 20,000 ppm in males, up to 11%), phospholipids (at 10,000 and 20,000 in males, up to 11%), triglycerides (at 20,000 ppm in both sexes, up to 47%) and ALT (at 20,000 ppm in both sexes, up to 48%) were dose-dependent and consistent with similar decreases in the 90-days study. LDH in females was dose-dependently reduced by up to 70% (statistical significance only in the high dose group). These statistically significant differences relative to controls were in part slight and showed no clear dose-response relationship and were not clearly related to the administration of the test material. Other statistically significant clinical chemistry findings (decrease in potassium in males at 20,000 ppm; increases in sodium and chloride, and a decrease in calcium in females at 20,000 ppm) were likewise also concluded in the study summary report not to be related to the administration of the test material.

Urine pH was significantly lower at 10,000 and 20,000 ppm but not significantly changed in the 5,000 ppm groups of males and females. The study report summary states that this might be due to urinary excretion of 2-phenoxyacetic acid, the major metabolite of 2-phenoxyethanol.

Statistically significant increases in relative organ weights at 10,000 ppm in males were observed in testes (+31%), lungs (+25%), kidneys (+16%) and brain (+16%) compared with controls. Comparable or higher increases at 20,000 ppm indicate that these increases were dose-dependent. These changes are likely to be related to the decrease in body weight observed in both sexes in this study. No increase in absolute organ weights was observed for adrenal or testis. Absolute organ weights were statistically significantly decreased in the heart and brain in males. Kidney weights were statistically significantly increased in males at ≥ 10,000 ppm but decreased in females at 20,000 ppm.
Relative liver weights were increased but absolute liver weights were decreased in males at ≥ 10,000 ppm. Absolute lung weights were increased in males at 20,000 ppm and relative lung weights were increased in males at ≥ 10,000 ppm. No histopathological changes were observed in kidney, liver, or lung in either sex. These organ weight changes were considered to be of no toxicological significance.

There was no evidence of a treatment-related increase in neoplastic lesions and no evidence of treatment-related non-neoplastic histopathological findings in either sex in this study.

Conclusions
There was no evidence of carcinogenic activity of 2-phenoxyethanol in male or female mice. No treatment-related histopathological findings were observed in any of the tissues evaluated in either males or females. Changes in clinical chemistry parameters that may suggest a treatment-related effect on the liver included decreases in cholesterol and phospholipids at ≥ 10,000 ppm and a decrease in triglycerides at 20,000 ppm in males. In the study summary report, these effects were concluded not to be clearly related to the administration of the 2-phenoxyethanol. There was no evidence of any histopathology in the liver and no increase in liver enzymes (ALT, AST). However, similar effects on cholesterol and phospholipids were observed in the 13-week drinking water study in the same strain of mice and these were very conservatively used to establish the NOAEL for that study. Therefore, these findings in males in the chronic study were also conservatively concluded to be treatment related. Furthermore, the significant effects on body weight gain indicate that the maximum tolerated dose was exceeded in males at 10,000 ppm in males (body weight 84% of controls) and in females at 20,000 ppm (body weight 79% of controls). No statistically significant changes of organ weights or biochemistry parameters were observed at the low dose (5,000 ppm). Therefore, the no-observed-adverse-effect level (**NOAEL**) for 2-phenoxyethanol in this study was concluded to be 5000 ppm in drinking water,

corresponding to an intake of **468 mg/kg bw/day** in males and **586 mg/kg bw/day** in females.

Subm II, ref. 5

3.3.8 Reproductive toxicity

3.3.8.1 Two-generation reproductive toxicity

Guideline:	Reproductive Assessment by Continuous Breeding (RACB); protocol designed by the NTP
Species/strain:	Mouse/CD-1
Group size:	40 breeding pairs (continuous breeding phase); 20 breeding pairs (crossover mating trial)
Test substance:	2-Phenoxethanol
Batch No.:	C093082 / 01
Purity:	94-95 %
Dose level:	0, 0.25, 1.25, 2.5 % , corresponding to approximately 400, 2000, and 4000 mg/kg bw/day in males. Daily doses in breeding females calculated from body weight and feed consumption data were approximately double those in males.
Route:	Oral, via diet
Vehicle:	Diet
Dosing schedule:	See summary text below
GLP:	Yes
Study Period:	13 Jul 1983 - 28 Mar 1984 (experimental phase)
Report Date:	1984

2-Phenoxyethanol was evaluated in a study conducted by the US National Toxicology Program (NTP) utilising the reproductive assessment by continuous breeding protocol designed by the NTP. Because the RACB protocol differs from OECD Guideline 416, it is described here. The protocol is divided into four tasks as described below.

Task 1: 14-day dose setting study (range finder)

Task 2:
Both sexes are dosed for 7 days prior to and during a 98-day cohabitation period. Animal pairs produce multiple litters during this period. Endpoints include clinical signs, parental body weight, fertility, and food consumption. The concentration of 2-phenoxyethanol in feed was 0, 0.25, 1.25, and 2.5%, corresponding to doses of approximately 400, 2000 and 4000 mg/kg bw/day in males based on the reported average daily feed consumption of 5.6 g/day in males (*Ref 14*). In females, average feed intake measured during week 18 of the study was 18.2, 17.2, 18.1, and 12. 1 g/day for the control, 0.25, 1.25, and 2.5% dose groups, respectively, and mean female body weights during this week were reported to be 44.4, 45.0, 48.5, and 40.5 g for the control 0.25, 1.25, and 2.5% dose groups, respectively (*Ref 15*). Taking these values to calculate exposure to 2-phenoxyethanol in the breeding females during that week of the study, the average estimated exposure in females was approximately 950, 4700, and 7500 mg/kg bw/day for the 0.25, 1.25, and 2.5% dose groups, respectively. Variability in actual intake in females can be expected to be greater than in males due to fluctuations in body weight based on stage of gestation. However, these values calculated for reproducing females during week 18 of the study are approximately two times higher than the intakes calculated for males.

Task 3:
When a positive effect on fertility is seen in Task 2, a 1-week crossover trial is conducted in which 20 pairs of parental animals (F0) per treatment group are mated for 7 days or until a copulatory plug is detected. The three treatment groups include: control males x control

females, control males x high-dose females, and control females x high-dose males. Treatment is discontinued for all animals during this week then reinstated at the appropriate dose until necropsy (3 weeks after the 7-day cross-over period). At the end of Task 3, F0 males and females are necropsied; endpoints evaluated are selective organ weights, body weight, epididymal sperm motility, morphology and number, and oestrous cyclicity as monitored by vaginal lavage for the preceding 7 days. Selected organs are evaluated for histopathology.

Task 4:
This is conducted whether or not Task 2 shows reproductive toxicity. The last litter from Task 2 is nursed, weaned, reared to sexual maturity while housed by sex, two or three per cage, and exposed to the same concentration of the test materials as their parents. At 74 ± 10 days of age, males and females from different litters within the same treatment group are cohabited for 7 days or until copulatory plug is seen and then housed individually until delivery. At the end of Task 4, the F1 mice are sacrificed and necropsied.

Results
2-Phenoxyethanol exhibited low general toxicity during the continuous breeding phase, with the high dose showing a minimal (2%) decrease in body weight in males while female body weights were unaffected. There was no change in the ability to produce multiple litters. There was no significant effect on feed consumption during this phase. There was no effect of 2-phenoxyethanol exposure on the number of pairs able to produce at least one litter (fertility index) or on the ability to produce multiple (five) litters during the continuous breeding period. At the high dose, exposure to 2-phenoxyethanol did significantly reduce the litter size, proportion of pups born alive and pup body weights during the continuous breeding period.
The cross-over mating trial indicated that neither mating index (percent copulatory plug positive matings) nor fertility index (fertile pairs) were altered by 2-phenoxyethanol pre-treatment. However, pup body weight was significantly decreased (12%) in the control male x 2.5% female mating group. At necropsy 3 weeks after the crossover mating trial, there was a significant decrease in body weight for F0 males but not for F0 females at the high dose. Liver weights were increased at the high dose in both males and females. Testis, prostate, and epididymal weights and sperm parameters were unaffected in males in the high dose.

To assess the reproductive effects on the F1 generation, the final continuous breeding litters from all dose groups were weaned at 21 days of age and 8 to 10 litters per group were randomly selected for rearing and subsequent breeding. Body weight at birth, weaning and mating showed dose-related decreases indicating treatment-related toxicity during the lactation and post-weaning periods. Dose-related pup lethality was also noted at the mid and high doses. Because of the high lethality rates in the high dose F1 animals, F1 matings were conducted only with control and mid-dose groups. Continuous exposure of the F1 mice to 1.25% in diet (indirectly *in utero* and during lactation and directly from weaning to 74 + 10 days of age) had no effect on mating index, fertility index, pups born live, or number of pups per litter. As observed for the F0 pairs, live pup weight (F2 pups) was decreased for the F1 pairs.

Conclusion
In this study, fertility was only minimally affected at the highest dose, but evidence of significant toxicity to the offspring was observed when 2-phenoxyethanol was administered at 1.25% and 2.5% in diet. The cross-over mating trial suggested a female component to the reproductive toxicity observed in high-dose females. The authors concluded that for male mice (F0) the **NOAEL** for reproductive toxicity was 2.5% in diet, corresponding to **4000 mg/kg bw/day**, and this occurred in the presence of evidence of parental toxicity (decreased body weight and increased liver weight). For both males and females, the NOAEL for parental toxicity and reproductive toxicity was concluded to be the low dose, i.e., 0.25% in diet. For males, a **NOAEL** of **400 mg/kg bw/day** was calculated. The estimated

corresponding daily intake of 2-phenoxyethanol in females calculated from average body weight and average feed consumption reported during week 18 was approximately **950 mg/kg bw/day**.

Ref.:14, 15

3.3.8.2 Other data on fertility and reproduction toxicity

No data available.

3.3.8.3 Developmental Toxicity

Study No 1 in rats

Guideline:	According to OECD guideline no. 414 (EC method B.31 (2004))
Species/strain:	Rat/Wistar
Group size:	25 females
Test substance:	2-Phenoxyethanol
Batch No.:	41183068E0
Purity:	>99.9% core peak area
Dose level:	0, 100, 300 and 1000 mg/kg bw/day
Route:	Oral, gavage
Vehicle:	Tap water with a few drops of Tween 80
Dosing schedule:	Days 6 through 19 of gestation
GLP:	Yes
Study Period:	27 Sep 2005 to 18 Oct 2005 (experimental phase)
Report Date:	December 2006

Samples of the dosing formulations were analysed for actual concentrations at the beginning and towards the end of the study. Stability and homogeneity were verified.
All animals were checked on a regular basis for mortalities and clinical signs, also food consumption was recorded on regular basis. At day 20 of gestation, all mated females were sacrificed. The scope of examinations in this study exceeded the OECD test guideline requirements with respect to haematology and clinical chemistry. At study termination on day 20 p.c. and following blood sample collection, the animals were sacrificed. Post mortem examination, including gross macroscopic examination of all internal organs, was performed and the ovaries and uteri were removed for the following examinations: gravid uterine weight (except for the prematurely sacrificed animal), number of corpora lutea, number of implantations (implantation sites, resorptions). The conception rates as well as the pre- and post-implantation losses were calculated.

Fetal evaluations included litter size, number of dead foetuses, foetal weight, sex ratio, and external malformations. The viability of the foetuses and the condition of the placentae, umbilical cords, foetal membranes and fluids were examined. Individual placental weights were recorded. Half of the foetuses were fixed in ethyl alcohol and stained for skeletal examination and the other half were fixed in Harrison's fluid for examination of any visceral findings.

Results
Dosing solutions were analysed twice during the duration of the dosing period. All measured individual values were within the required range of ±10% of the nominal concentration.
One animal in the high-dose group was prematurely sacrificed in a moribund state. Test substance-related clinical signs of maternal toxicity were observed at the high dose (1000 g kg bw/day). One dam at this dose was sacrificed in moribund condition on day 14 p.c., which was considered as treatment-related. All dams showed unsteady gait and transient salivation for a time of up to 3.5 hours after dosing at least once during the treatment period. A few times, high-dose rats were found in lateral position shortly after treatment, had vaginal haemorrhage and/or urine smeared fur. At this dose level, food consumption as

well as gross and net body weight gain were clearly affected by the test compound. There were no clinical signs of test substance-related maternal toxicity in the mid- or low-dose dams.

No treatment-related changes were noted in haematology or serum enzymes. Clinical chemistry investigations showed decreased concentrations in total bilirubin (-31%), total protein (-7%), albumin (-6%) and globulin (-8%) as well as increased triglyceride values (+50%) in the serum of the high-dose animals (1,000 mg/kg bw/day) with statistically significant differences compared to the control group. Somewhat lower but also statistically significant changes were noted in the mid-dose animals (300 mg/kg bw/day): total bilirubin (-21%), total protein (-2%), albumin (-4%) as well as increased triglyceride values (+40%). The total protein (sum parameter including globulin) results were slightly below the lower limit of historical controls. Whereas liver enzymes in serum were not influenced, all of these findings were related to adaptational metabolic changes of liver functions rather than to particular organ toxicity. Although these effects were likely to be caused by the test material, they were not considered as adverse *per se*. The other clinical chemistry changes are not considered treatment-related.

There were no differences of toxicological relevance between the control and the test substance-treated groups in conception rate, mean number of corpora lutea, total implantations, resorptions and live foetuses, foetal sex ratio, or in calculated pre- and post-implantation losses.
No test substance-related differences were recorded for placental and foetal body weights. The external and soft tissue and/or skeletal examinations of the foetuses revealed no toxicologically relevant differences between the control and the treated groups. The number and type of foetal external, soft tissue and skeletal findings, which were classified as malformations and/or variations, did not show differences of toxicological relevance among the groups.

Conclusion
Based on the effects in the dams observed at 1000 mg/kg bw/day, the **NOAEL** (no-observed adverse-effect level) for maternal toxicity was considered to be **300 mg/kg bw/day**. 2-Phenoxyethanol had no effect on gestational parameters and induced no signs of developmental toxicity up to and including the high dose of 1000 mg/kg bw/day. Therefore, the **NOAEL** (no observed effect level) for developmental toxicity was considered to be **1000 mg/kg bw/day**.

Ref.: 16

Study No 2 in rabbits (dose-range finding study)

Guideline:	/
Species/strain:	Rabbit/New Zealand White
Group size:	10 females
Test substance:	2-Phenoxyethanol
Batch No.:	53 (C44172)
Purity:	99.9%
Dose level:	0, 300, 600 and 1000 mg/kg bw/day
Route:	Dermal, occlusive conditions (24 hours/day throughout the treatment period)
Vehicle:	Undiluted
Dosing schedule:	Days 6 through 18 of gestation
GLP:	No information
Report Date:	1985

A dose-range finding study was conducted to establish the maximum-tolerated dose level of 2-phenoxyethanol via dermal application in pregnant rabbits. Prior to each application, the

skin at the application site was examined for signs of irritation. All rabbits were sacrificed on day 19 of gestation.

Results
After dermal application of 1000 mg/kg/day of 2-phenoxyethanol to pregnant rabbits, a statistically significant weight loss was seen from day 15 through 18 of gestation. In both the mid- and high-dose group, one animal was sacrificed in a moribund condition. In the high-dose group, the moribund condition of the rabbit was attributed to a hairball in the stomach. The rabbit in the mid-dose group was found to have an intussuception (congestion) of the colon. No evidence of maternal toxicity was observed in rabbits dermally given 300 or 600 mg/kg/day of 2-phenoxyethanol; no evidence of embryonal toxicity was observed at any dose level. Due to rubbing of the bandage where it was taped in place, some evidence of focal irritation to the skin was observed upon gross examination of rabbits at all dose levels including controls. Based on the results of this probe study, and the physical constraints of applying more than 1 ml/kg of 2-phenoxyethanol, 1000 mg/kg/day was considered slightly toxic to the maternal animals and dose levels of 300, 600 and 1000 mg/kg/day were selected for the definitive dermal teratology study in this species.

Ref.: 17

Study No 3 in rabbits

Guideline:	/
Species/strain:	Rabbit/New Zealand White
Group size:	25 females
Test substance:	2-Phenoxyethanol
Batch No.:	not reported (probably 53 (C44172) as in the probe study above)
Purity:	>99%
Dose level:	0, 300, 600 and 1000 mg/kg bw/day
Route:	Dermal, occlusive conditions (24 hours/day throughout the treatment period)
Vehicle:	Undiluted
Dosing schedule:	Days 6 through 18 of gestation
GLP:	No information
Report Date:	1985 (study published in 1987)

In the main study, groups of 25 artificially-inseminated female rabbits were treated with 300 600, or 1000 mg/kg bw/day of undiluted 2-phenoxyethanol, applied to shaved skin, on days 6 through 18 of gestation. The dose volumes of undiluted 2-phenoxyethanol (volume weight 1.1 g/ml) were 0.27, 0.55, and 0.91 ml/kg bw, respectively. Control animals were treated with distilled water at a dose volume of 0.91 ml/kg bw. The application site was occluded. Bandages remained in place 24 hr per day throughout the treatment period. Prior to each application, the skin at the application site was examined for signs of irritation. On day 19, bandages were removed and the application site was washed with water to remove any residue of test material to prevent oral ingestion.

Pregnant animals were observed daily for evidence of treatment-related effects. Statistical analysis of body weight and body weight gain were performed using data recorded on gestation days 6, 9, 12, 15, 19 and 28. Blood was collected from an ear vein from approximately 10 animals per dose group (control, 300, and 600 mg/kg bw/day) on day 19 of gestation and 3 animals (2 at 600 mg/kg bw/day and 1 at 1000 mg/kg bw/day) sacrificed *in extremis* for measurement of haematological parameters including packed cell volume (PCV), haemoglobin (HGB), erythrocyte count (RBC), total leukocyte count (WBC), red blood cell indices (MCV, MCM, MCHC), reticulocyte count, red cell osmotic fragility, WBC differential counts and platelet count. Urine was collected at necropsy from 2 moribund rabbits (one each at 600 and 1000 mg/kg bw/day) via aspiration for urinalysis. Maternal liver weights were recorded at the time of Caesarean section on day 28.

Following Caesarean section, the number of *corpora lutea* and the number and position of implantations, resorptions and live or dead foetuses were recorded. The uteri of apparently non-pregnant females were stained and examined for evidence of early implantation sites. Foetal examinations included litter size, number of dead foetuses, foetal body weights, crown-rump length measurement, sex determination and external alterations. One-half of each litter were examined under a dissecting stereomicroscope for evidence of visceral alterations. All foetuses were preserved and examined for skeletal alterations.

Results
Throughout the dosing period, slight to moderate reddening at the application site was seen in some animals at all treatment levels. No treatment related changes of maternal body weight, body weight gain or liver weights were observed. Maternal toxicity as evidenced by intravascular haemolysis of red blood cells and death was seen in pregnant rabbits treated with 600 and 1000 mg/kg bw/day. Maternal toxicity was dose related, with 9 dead or moribund animals at 1000 mg/kg bw/day, 5 at 600 mg/kg bw/day, and no deaths at 300 mg/kg bw/day. Most deaths occurred between gestation days 11 and 18 (6 to 13 doses). Deaths occurred rapidly after the onset of clinical signs, usually within 24 hrs. With the exception of 5 animals that had already survived to day 28 due to staggered initiation, all remaining animals of the 1000 mg/kg bw/day group that survived to day 18 were killed on this day for humane reasons with no further observations. Intravascular haemolysis was diagnosed in moribund animals based on changes in haematology including severely depressed RBC counts and PCV values as well as elevated reticulocytes and increased red blood cell fragility. Dark urine in these animals was concluded to be due to haemoglobinuria. Animals in the 600 (N = 20) and 1000 mg/kg bw/day (N = 5) groups that survived to day 28 of gestation had no evidence of treatment-related effects, including no evidence of intravascular haemolysis. No signs of maternal toxicity were seen at 300 mg/kg bw/day. No differences in body weight gains or absolute or relative liver weights were observed between treated rabbits and controls.

The number of foetuses (and litters) externally examined from the control, 300, 600 and 1000 mg/kg bw/day/ groups were 128 (17), 142 (20), 136 (15) and 49 (5), respectively. Treatment with 300 or 600 mg/kg bw/day had no adverse effect on the pregnancy rate, the number of *corpora lutea*, implantations, resorbed implantations, or live foetuses per litter or foetal body measurements. Inadequate numbers of surviving dams at 1000 mg/kg bw/day precluded a full evaluation of developmental toxicity at 1000 mg/kg bw/day although no evidence of adverse effects in foetuses from 5 surviving animals was seen.
Treatment with up to 600 mg/kg bw/day had no effect on the incidence or type of external, visceral or skeletal malformations. Single occurrences of hemivertebrae (one in the mid-dose group) and clinodactyly (curved toe bone; one each in the low- and mid-dose group) were observed among litters of dosed animals. The low incidences of these malformations were considered to be of sporadic occurrence and not indicative of a treatment-related effect. One control group foetus exhibited oligodactyly. Foetuses from the 5 animals treated with 1000 mg/kg bw/day that survived to day 28 also did not exhibit external, visceral or skeletal alterations (no statistical evaluations were performed on these data).

Conclusion
Dermal application of 2-phenoxyethanol produced no evidence of teratogenicity, fetotoxicity or embryotoxicity under the conditions of this study. Maternal toxicity, including dose-related intravascular haemolysis, was observed in dams dosed with 600 and 1000 mg/kg bw/day. The **NOAEL** for maternal toxicity was considered to be **300 mg/kg bw/day.** The **NOAEL** for developmental toxicity was concluded to be **600 mg/kg bw/day** because maternal deaths at 1000 mg/kg bw/day precluded a full evaluation of developmental toxicity in the highest dose group.

Ref.: 18

SCCS Comment
The report of the original main study (Scortichini et al. 1985, AR 6) was not provided; only a published report is available (ref. 18). Breslin et al. (1991, ref. 12), the authors of the 90-day dermal toxicity study in rabbits, reported that according to their experience from additional pilot studies conducted with non-pregnant rabbits at dermal doses of 1000 mg/kg bw/day for 14 days, the susceptibility of individual rabbits to the haemolytic effects at high doses of dermally applied 2-phenoxyethanol was variable, with many animals exhibiting no haematolytic effects. However, once clinical signs developed, death followed rapidly, usually within 24 hrs (Phillips et al. 1985 (subm II, ref. 1; Kirk et al, 1985, AR 7, quoted in Phillips et al. 1985 (subm II, ref. 1) and in ref. 18).

3.3.9 Toxicokinetics

3.3.9.1 Toxicokinetics in vitro

***In vitro* metabolism of 2-Phenoxyethanol with S9 Homogenates of Rat Liver and Skin**

Incubation on 2-phenoxyethanol with rat liver and skin post-mitochondrial fraction resulted in the formation of 2-phenoxyacetic acid. During the 60-minute incubation, the rate of formation of 2-phenoxyacetic acid after 1 hour was 147 nmol/g skin and 2900 nmol/g liver. The skin had the potential to metabolise 2-phenoxyethanol at a rate of approximately 5-7% of the liver. Metabolism was inhibited by 1 mM pyrazole, suggesting involvement of alcohol dehydrogenase. The failure to detect first pass metabolism to 2-phenoxyacetic acid in the percutaneous penetration experiments may have been the result of competition for the enzyme due to the use of methanol as vehicle. Also, the high rate of penetration may have reduced the opportunity for first-pass metabolism of 2-phenoxyethanol in the skin in this study.

Ref.: 9

***In Vitro* Metabolism of 2-Phenoxyethanol in Liver S9 Homogenates from Rat, Mouse, Rabbit and Human**

Guideline:	/
Species/Strain:	Liver S9 homogenate prepared from female Sprague-Dawley rats, CD-1 mice, New Zealand White rabbits and human donors (Caucasians)
Replicates:	Probe study, Phase I, and Phase II: 2 replicates Phase III: 3 replicates
Test substance:	2-Phenoxyethanol
Batch No.:	QF1750UKAO
Purity:	99.7%,
Test concentration:	Probe study: 33 µg/mg protein/ml Phase I: 0.07, 0.22 0.72, 2.2, 7.2 and 22 µmol/mg protein/ml Phase II: 10 and 30 µg/mg protein/ml Phase III: 10, 33, 80, 160, 300, and 1000 µg/mg protein/ml (nominal)
Stability:	Overall mass balance of substrate (2-phenoxyethanol) plus metabolite (2-phenoxyacetic acid) averaged 100.3% after incubations of 1, 2, 5, 10, 20, 60, and 120 min
Incubation time:	Probe study: 120 min. Three experiments were made: Phase I: 120 min Phase II: 0, 1, 5, 10, 20, 60, and 120 min Phase III: 0, 120 min

GLP: Yes
Study Period: 24 Jul 2006 – 31 Jul 2006
Report Date: 2006

In a special test design, the *in vitro* metabolism of 2-phenoxyethanol (purity 99.7%) in liver S9 homogenates of female mice, female rats, female rabbits, and female human donors was compared. The levels of parent compound and known metabolites, 2-phenoxyacetic acid (PAA) and phenol were determined in the liver S9 homogenate (HPLC/UV). Several substrate concentrations of 2-phenoxyethanol were incubated with S9 homogenate for various times to determine apparent rate constants (Km, Vmax) for metabolism of this test material.

The probe experiment evaluated the primary enzyme system(s) responsible for 2-phenoxyethanol metabolism *in vitro* with liver S9 homogenate, a probe experiment was conducted utilising either NAD^+ or NADPH cofactors. Each cofactor (1 mM; NADPH samples also had 3 mM $MgCl_2$) was incubated in duplicate with liver S9 fractions from female mice, female rat, female rabbit and female human donors. The samples were incubated in 0.1 M potassium phosphate buffer with a nominal concentration of 33 µg 2-phenoxyethanol/mg protein/ml of incubate for 2 hours at 37 °C (1 ml final sample volume). After 2 hours of incubation at 37 °C, the reaction was stopped by addition of acetonitrile. The samples were analysed via HPLC and the amount of 2-phenoxyethanol and metabolites (PAA and phenol) were determined.

Phase I experiment:
Phase I evaluated the relative rates of 2-phenoxyethanol metabolism in the S9 liver fractions of female rats by determining the disappearance of parent compound and the formation of metabolites (PAA and phenol) in liver S9 incubations for 2 hours at six substrate concentrations. Duplicate liver S9 samples were incubated in the presence of 2-phenoxyethanol at 0.07, 0.22, 0.72, 2.2, 7.2, and 22 µmol/mg protein/ml (0.01, 0.03, 0.1, 0.3, 1, and 3 mg/ml) at 37 °C in a shaking bath and 1 mM NAD^+ (as determined in the Probe Study) (1 ml final sample volume).
Incubations were stopped at 120 minutes by adding a sufficient amount of acetonitrile containing 5% acetic acid. The concentration of remaining 2-phenoxyethanol was determined and plotted against the original concentration of 2-phenoxyethanol (as determined at T=0). The linear range of 2-phenoxyethanol metabolism was then determined.

Phase II:
The Phase II was designed to evaluate the relative rates of 2-phenoxyethanol metabolism in liver S9 fractions of mice, rats, rabbits, and human donors by determining the levels of parent compound and known metabolites (PAA and phenol) in liver S9 incubations at two concentrations over several incubation time periods. Duplicate liver S9 samples were incubated in the presence of 2-phenoxyethanol, at two concentrations as determined in Phase I, at 37 °C in 0.1 M potassium phosphate buffer for 0, 1, 5, 10, 20, 60, and 120 minutes in a rotating water bath and necessary cofactors were added to initiate the reaction (1 ml final sample volume). The incubations were stopped at the designated time points by adding a sufficient amount of acetonitrile containing 5 % acetic acid.

Phase III:
Triplicate liver S9 fractions from mouse, rat, rabbit, and human donors were incubated in 0.1 M potassium phosphate buffer (1 mM NAD^+) at nominal 2-phenoxyethanol concentrations of 10, 33, 80, 160, 300, 1000 µg/mg protein/ml incubate (1 ml final sample volume). Concentration of 2-phenoxyethanol, PAA, and phenol were determined via HPLC at T=0 and at T=120 minutes. From these data, apparent Km and Vmax values were determined for each S9 type.

Results
Incubation with the NAD+ cofactor afforded the highest amount of 2-phenoxyethanol conversion. The metabolic loss of substrate in liver S9 was primarily NAD+-dependent, indicating metabolism of 2-phenoxyethanol primarily via alcohol and aldehyde dehydrogenases. The major metabolite identified was 2-phenoxyacetic acid (PAA), accounting for >25 % at the lower substrate concentrations in rat and human liver homogenate. Phenol was found as a minor metabolite only in incubations of liver S9 homogenate from female mice utilising NADPH as a cofactor.
The formation of PAA was found to saturate at 0.8-2.5 mM (product concentration ~ 0.09 mM). Metabolite formation was found to be linear up to 120 minutes. The overall mass balance of substrate in the incubations (final 2-phenoxyethanol + PAA concentrations / initial 2-phenoxyethanol concentration) averaged 100.3%. The rate of 2-phenoxyethanol metabolism at the substrate concentration of ~1 mM was found to be highest in the human > rat > mouse > rabbit. The intrinsic clearance (V_{max}/K_m) calculated for 2-phenoxyethanol from these data were human > rat > mouse (0.01225, 0.00199 and 0.00129 ml/min/mg protein, respectively). The observed V_{max} of 0.00011 µmol/min/mg protein for rabbit liver S9 was seen at the lowest substrate concentration of 0.07 mM.

Conclusion
The rate of metabolism of 2-phenoxyethanol to 2-phenoxyacetic acid was highest in liver S9 homogenates from human female donors followed by rat > mouse > rabbit.

Ref.: 26

SCCS Comment
Due to a limitation of the substrate concentrations in the lower range, the intrinsic clearance (V_{max}/K_m) of rabbit liver S9 could not exactly be determined. If K_m is assumed to be around 50% of the lowest substrate concentration of 0.07 mM (a conservative assumption), then V_{max}/K_m ≈0.00011 / 0.035 ≈ 0.0031 ml/min/mg protein, i.e. the intrinsic clearance of rabbit liver S9 is probably in the same order of magnitude as that of human, rat and mouse liver S9.
However, the data suggests low-capacity and high-affinity enzyme activity of rabbit liver S9 at the rate-limiting enzymatic step compared with human, rat and mouse liver S9. Due to potential saturation of metabolising enzyme(s) at lower substrate concentrations in the liver of rabbits compared with humans, rats or mice, rabbit might be a more sensitive species to high doses of 2-phenoxyethanol than human, rat and mouse.

3.3.9.2 Toxicokinetics in laboratory animals

Absorption, distribution, elimination and kinetics of ^{14}C-2-Phenoxyethanol in rats after oral and dermal exposures

Howes (1989) reported studies on the fate of 2-phenoxyethanol in rats after dermal and oral application. Groups of 3 to 4 male or female Wistar rats (about 150 g bw) were treated by gavage with an aqueous solution of 2-phenoxy[1-^{14}C]ethanol at doses of 16 to 160 mg/kg bw (radiochemical purity >99%). The test substance was completely absorbed and rapidly excreted. >90% of ^{14}C was found in urine where only 2-phenoxyacetic acid and its derivatives, mainly conjugates, could be detected. Whereas 1-2% of ^{14}C equivalents were exhaled as radiolabelled ethanol, around 1% was found in the tissues after 4 days. Faeces also contained around 1% of ^{14}C. The overall disposition of ^{14}C equivalents was not influenced by the size of the dose or gender of the rats.

Male and female rats were treated topically with 2.8 or 3.7 mg 2-phenoxy[1-^{14}C]ethanol dissolved in ethanol (corresponding to 20-25 mg/kg bw). Female rats were treated with one of two skin creams containing 1% ^{14}C-2-phenoxyethanol (about 1 mg corresponding to about 7 mg/kg bw). Whereas ethanol as solvent is not ideal because it may interfere with oxidative metabolism, 2-phenoxyethanol was rapidly absorbed and mainly excreted in urine

(55-60%). Proportions of ^{14}C exhaled (1 - 1.5%) or found in tissues (1 - 2%) or faeces (~2%) were similar to the gavage experiments. Recovery, however, was only around 70%, probably due to evaporation from skin when dissolved in ethanol and applied. Some 88% of the test substance applied in skin creams was absorbed and recoveries were >90%.
Radiolabelled substances in rat urinary samples were separated by conventional techniques and identified by MS. Around 78% were identified as 2-phenoxyacetic acid. After acid hydrolysis of conjugates, 2-phenoxyethanol was the main constituent of the remaining 22% ^{14}C, but the exact amounts could not accurately be quantified. The residual substances constituted ring-hydroxylated metabolites of 2-phenoxyethanol and 2-phenoxyacetic acid.

In conclusion, after dermal or oral exposure of rats, most of radiolabelled 2-phenoxyethanol was rapidly excreted in urine. Distribution was apparently independent of the dose (up to 160 mg/kg), gender or exposure route. After oral exposure, only free 2-phenoxyacetic acid and to a minor extent its conjugates were found in urine. After dermal exposure, mainly free 2-phenoxyacetic acid, and to a minor extent (22%) conjugates of 2-phenoxyethanol and 2-phenoxyacetic acid, were found in urine. Ring-hydroxylated conjugates of 2-phenoxyethanol and 2-phenoxyacetic acid were also detected. Data in rats suggest higher systemic availability of 2-phenoxyethanol after dermal exposure than after oral exposure.

Subm II, ref. 6 and 7

Metabolites in rabbits after oral exposure

Breslin et al. (1991) reported the occurrence of free 2-phenoxyethanol and free 2-phenoxyacetic acid (PAA) in serum after oral exposure of female rabbits with 800 mg/kg bw 2-phenoxyethanol. The substance appeared to be rapidly metabolised as high levels of PAA were observed in serum samples at all time periods. Peak PAA concentrations (1452 µg/ml) occurred 3 hr after dosing. Lower levels (0-25 µg/ml) of 2-phenoxyethanol were observed in all serum samples, i.e. up to about 1.6% of the bioavailable dose in serum.
In preliminary analyses of serum samples taken from rabbits treated by gavage with 600 mg 2-phenoxyethanol/kg/day for 2 consecutive days, trace concentrations of the metabolite phenol (<1 µg/ml) were observed. In addition, approximately 90% of 2-phenoxyethanol and phenol and 50% of PAA observed in serum were glucuronide or sulfate conjugates. Metabolites in urine were not investigated.
In conclusion, metabolism to PAA appeared to be slower than in rats (peak concentration after 3 hrs compared to T_{max} of 1-2 hrs in rats) and up to 16% of the bioavailable dose in serum consisted of free and conjugated 2-phenoxyethanol, i.e. more than in rats (0.07% of the dose; see ref. 27 and ref. 28, just below).

Ref. 12

Absorption, distribution, elimination and kinetics of ^{14}C-2-Phenoxyethanol in rats after oral exposure

Guideline:	According to OECD Guideline no. 417
Species/strain:	Rat/Wistar
Group size:	Experiments 1-7 and 10: 4 animals per sex per dose; Experiments 8 and 9: 3 females per time point; Experiment 11: 4 females
Test substance:	Protectol® PE (2-Phenoxyethanol)
Batch:	41183068E0
Purity:	>99.9% (GC)
Test substance:	2-hydroxyethyl-[phenyl-U-^{14}C]ether (^{14}C-2-phenoxyethanol)
Batch:	873-1012
Purity:	Radiochemical purity 97.9% (radio-HPLC); Chemical purity 96.5% (HPLC)
Specific Activity:	6.7 MBq/mg (LSC measurement)
Vehicle:	0.5% Carboxymethylcellulose
Dose levels:	Experiments 1-4: 30, 100, 300, or 1000 mg/kg bw single oral dose;

	Experiments 5-7: 40 or 400 mg/kg bw as a single dose and 400 mg/kg bw/day for multiple doses; Experiments 8 and 9: 40 or 400 mg/kg bw; Experiments 10 and 11: 40 or 400 mg/kg bw
Dose volume:	10 mL/kg bw
Route:	Oral (gavage)
Dosing schedule:	Experiments 1-6, 8-11: single dose; Experiment 7: multiple dose (daily oral dosing with unlabelled test substance for 14 days and one dose of labelled test substance on Day 15)
GLP:	Yes
Study Period:	02 Dec 2005 – 28 Jun 2006
Report Date:	2007

^{14}C-2-Phenoxyethanol and the non-radiolabelled test substance were prepared in 0.5% carboxymethylcellulose in double distilled water. In order to achieve the required specific activity, respective amounts of non-radiolabelled material were added to the respective amounts of stock solution of radiolabelled material and the aqueous vehicle was added and filled up to the final volume. The required quantity of radioactivity per animal was about 0.5 to 2.5 MBq.

Experiments 1 to 4:
In male and female rats, kinetic parameters were determined based on total radioactivity following a single oral dose of ^{14}C-2-phenoxyethanol at four dose levels (30, 100, 300 and 1000 mg/kg bw). Blood samples were collected at 1, 2, 4, 8, 24 hr and subsequently in time intervals of 24 hr up to 96 hr.

Experiments 5 to 7:
The absorption, distribution and excretion of radiolabelled products were investigated after single (40 and 400 mg/kg bw) and multiple (one administration of unlabelled test item for 14 days and one of labelled test item on day 15) oral doses of ^{14}C-2-phenoxyethanol in male and female rats. Therefore, urine was collected after 6, 12, and 24 hr and subsequently in time intervals of 24 hr up to 96 hr. Faeces were sampled in intervals of 24 hr up to 168 hr. After 168 hr, animals were sacrificed and the following organs and tissues (heart, liver, spleen, bone, skin, lung, ovaries/testes, carcass, muscle, kidney, brain, pancreas, uterus, adipose tissue, stomach and stomach contents, thyroid glands, adrenal glands, blood cells and plasma, gut and gut contents, bone marrow) were evaluated for remaining radioactivity.

Experiments 8 and 9:
In order to determine the tissue distribution of radioactivity, female rats were administered a single oral dose of 40 mg or 400 mg ^{14}C-2-phenoxyethanol/kg bw. The same organs and tissues as in the experiments 5 to 7 were collected at four different time points, corresponding to the maximum plasma concentration (MPC), 1/2 MPC, 1/4 MPC and 1/8 MPC. For the high dose, samples were collected at 2, 4.5, 7 and 14 hr and for low dose at 1, 2, 3.5 and 8 hr.

Experiments 10 and 11:
Biliary excretion of radioactivity following a single oral dose of 400 mg ^{14}C-pheoxyethanol/kg bw was determined in male and female rats. In addition, females only were administered a single dose 40 mg/kg bw. Bile was collected in 3 hr-intervals, and urine and faeces were collected in 24-hr time intervals up to 72 hr. After 72 hr, stomach and stomach contents, gut and gut contents and carcass were evaluated for remaining radioactivity.

During the experiments 5 to 11, samples of urine, plasma, faeces, and bile were generated following administration of ^{14}C-2-phenoxyethanol, which were used for an investigation of

metabolism. The metabolism study (Ref. 28) is described in a separate study summary (see below).

Results
After a single oral dose of 40 mg/kg bw or 400 mg/kg bw of ^{14}C-2-phenoxyethanol, mean total recoveries of radioactivity were 97.55% in males and 96.26% in females. No relevant amount of the administered radioactivity was detected as CO_2 in exhaled air for either dose. At 168 hours after dosing with 40 mg/kg bw, the total amount of radioactivity excreted in urine was 94.0 and 92.9% in males and females, respectively. The corresponding values for animals dosed with 400 mg/kg bw were 94.1 and 93.4% in males and females, respectively. Within 168 hours after dosing with 40 mg/kg bw, 2.2 and 1.9% of the administered radioactivity was excreted via faeces in males and females, respectively. The corresponding values for animals dosed with 400 mg/kg bw were 2.9 and 2.0% in males and females, respectively. The time course for appearance of radioactivity in urine and faeces indicates rapid excretion after administration of both doses. The plasma kinetics are presented in **Table 4**.
Increasing the dose by a factor of 33 (from 30 to 1000 mg/kg bw) resulted in an increase in AUC values by a factor of 61 in males and 88 in females. These data indicate a saturation of excretion with increasing dose.

Table 4
Plasma kinetics in rat after oral exposure

Sex	Dose (mg/kg bw)	C_{max} (µg Eq/g)	T_{max} (hour)	Initial half-life (hour)	Terminal half-life (hour)	AUC (µg Eq*hour/g)
Male	1000	415.04	1	2.29	39.57	3459
	300	82.78	2	2.91	33.10	567
	100	47.95	1	1.92	54.05	207
	30	13.66	1	2.04	46.42	57
Female	1000	487.73	2	4.60	40.66	4596
	300	127.26	2	1.87	60.45	801
	100	54.41	1	1.82	32.15	238
	30	14.26	1	2.09	27.99	52

A comparable time course of radioactivity was found for blood and plasma in both sexes. During the first two days post-dosing, lower concentrations of radioactivity were generally found in the blood indicating that the majority of the radioactivity was in the plasma and not bound to cellular blood constituents. Blood/plasma ratios >1 were not detected within the first 24 hours after dosing, but were observed at 48 hours after dosing or later. Following a single oral dose of 400 mg/kg bw, tissue distribution of radioactivity was measured 2, 4.5, 7, and 14 hours post-dosing in females. Following a single oral dose of 40 mg/kg bw, corresponding measurements were performed 1, 2, 3.5, and 8 hours post-dosing in females. Tissue radioactivity in general declined with time parallel to plasma concentrations. Through the time course of the experiments, the highest radioactivity concentrations were found in the GI tract, kidney, pancreas, skin and bone marrow for the high dose as well as GI tract, kidney, liver, and skin for the low dose. Radioactivity levels were lowest in the brain, muscle, and heart for the high dose and the brain, uterus, muscle and bone for the low dose. Within 72 hours after administration of 400 mg/kg bw ^{14}C-2-phenoxyethanol, excretion via bile was found to be about 5.6% and 4.6% of the administered dose in male and female animals, respectively. Within 72 hours after administration of 40 mg/kg bw ^{14}C-2-phenoxyethanol, excretion via bile was about 3.4% of the administered radioactivity in female animals.

Conclusion
After a single oral administration, ^{14}C-2-phenoxyethanol was rapidly and almost completely absorbed from the gastrointestinal tract. After absorption, radioactive material was distributed in all organs and tissues. The excretion of radioactivity was very rapid and occurred mainly via the urine within 24 hr post dosing. Based on the amounts of radioactivity excreted via bile and urine, the high bioavailability of ^{14}C-2-phenoxyethanol was confirmed, since the sum of radioactivity excreted via bile and urine was over 90% of the recovered radioactivity in the bile experiments.
Within the current study, the bioavailability of ^{14}C-2-phenoxyethanol was comparable at the dose of 400 and 40 mg/kg bw. The plasma kinetics demonstrated that an increase of the dose resulted in a disproportional increase of the AUC values, indicating a saturation of excretion with increasing dose.

Ref.: 27

Absorption, metabolism and excretion of ^{14}C-2-Phenoxyethanol in rats after oral exposure

Guideline:	According to OECD Guideline no. 417
Species/strain:	Rat/Wistar
Group size:	4 Females in groups R, C, and D; for groups B, V, S, and W, four females were dosed, whereas samples from only 3 animals were analysed.
Test substance:	2-Hydroxyethyl phenyl ether (2-Phenoxyethanol)
Batch:	41183068E0
Purity:	>99.9% (GC)
Test substance:	2-hydroxyethyl-[U-14C]phenyl ether (^{14}C-2-phenoxyethanol)
Batch:	873-1012
Purity:	Radiochemical purity 97.9% (radio-HPLC); Chemical purity 96.5% (HPLC)
Specific Activity:	6.7 MBq/mg (LSC measurement)
Vehicle:	0.5% Carboxymethylcellulose
Dose levels:	Group B, 40 mg/kg bw; Group C, 400 mg/kg bw; Group D, 400
mg/kg	bw, Group R, 40 mg/kg bw; Group S, 400 mg/kg bw; Group V, 40 mg/kg bw; Group W, 400 mg/kg bw
Dose volume:	10 mL/kg bw
Route:	Oral (gavage)
Dosing schedule:	Groups B, D, R, S, V, and W: single dose; Group C: daily oral dosing with unlabelled test substance for 14 days and one dose of labelled test substance on Day 15
GLP:	Yes
Study Period:	27 Mar 2006 – 11 May 2007
Report Date:	2007

All samples analysed in this **metabolism study** were generated during the absorption, distribution, elimination and biokinetics study described above (Ref. 27; hereafter referred to as the **biokinetics study**). Groups B, C, D, R, S, V, and W corresponded to the animals from experiments 6, 7, 5, 11, 10, 9, and 8 from this biokinetics study. Biological samples taken for analysis included urine, faeces, plasma and bile.

Three dose groups (B, D, and C) were used in the biokinetics study to investigate the absorption, distribution, and excretion of ^{14}C-2-phenoxyethanol in male and female Wistar rats after oral gavage. Dose group B received a single oral dose of 40 mg/kg bw and dose group D received a single dose of 400 mg/kg bw. The latter dose group was used to generate sufficient material for isolating and identifying metabolites. Dose group C consisted of animals receiving a daily dose of 400 mg/kg bw unlabelled 2-phenoxyethanol for 14 days followed by ^{14}C-2-phenoxyethanol at a dose of 400 mg/kg bw. Excreta were collected for up to 7 days.

The dose groups R and S represented bile-canulated male and female rats that received a single oral dose of 40 mg/kg bw and 400 mg /kg bw for groups R and S, respectively. Bile was collected for a time period of up to 72 hrs.
Samples of urine and bile from selected time intervals for the above-mentioned dose groups were investigated for metabolites within the study summarised here. For investigations on tissue distribution, dose groups V (40 mg/kg bw) and W (400 mg/kg bw), rats received a single dose of the test compound and different samples were collected near the time point of maximum plasma level (T_{max}). Plasma samples taken at 1 hr and 2 hrs post-dose for dose groups V and W, respectively, were investigated for metabolite identification.
For all dose groups evaluated, samples from females animals only were analysed for metabolites.

Results

Plasma Metabolites:
In dose groups V and W, where plasma samples were taken at 1 hr and 2 hr, the dominant metabolite identified in plasma was M01, 2-phenoxyacetic acid. The absolute amount of M01 recovered in plasma was low (0.3% and 0.l% of the applied dose) but covered approximately 70-90% of the total radioactivity in plasma depending on the dose group. Additional peaks were detected at trace amounts (<0.05% of administered dose). Phenoxyethanol parent compound was not identified in any sample.

Urinary Metabolites:
Urine samples from female animals excreted within 0-6 hr and 6-24 hrs were analysed for metabolites. The metabolite M01, 2-phenoxacetic acid, was dominant throughout all dose groups, amounting to 58.6 – 63.7% of the applied dose within 24 hrs. According to the value of the corresponding region of interest within the 0-6 hr interval, the values of the applied dose were extrapolated for the complete sampling interval of up to 168 hrs. The extrapolation results indicated that 2-phenoxyacetic acid represented 61.0 – 68.8% of the applied dose. Therefore, the test material was mainly formed by oxidation of the terminal hydroxyl group to the corresponding carboxylic acid yielding the dominant metabolite M01.

The glucuronidated metabolite M05 was quantified for all dose groups and represented 4.8 – 6.0% of the applied dose during the first 24-hr sampling period. Additionally, two metabolite groups, M02/M03/M07 and M04/M08, were not separated by either of two HPLC methods initially used. The metabolites present in these co-eluting peaks were investigated using other HPLC methods and LC/MS/MS analysis. From these analyses, M02 was identified as a ring-sulfated product of 2-phenoxyethanol which comprised up to 85% of the radioactivity within the M02/M03/M07 co-eluting peak. The metabolites M04 and M08 were assumed to be present at approximately equal amounts, as assessed by LC-MS/MS analysis. These metabolites were either hydroxylated or sulphonated in the phenyl ring or both, and oxidized to carboxylic acid or glucuronidated at the side chain. They were detected at 8.0 – 10.3% (M02, M03 and M07) and 4.7 – 5.9% of the dose (M04 and M08) during the first 24 hr. A comparison of the retention times of the test item, 2-phenoxyethanol, and the corresponding peak in chromatograms from urine analyses indicated the presence of 2-phenoxyethanol in samples from the time periods 0-6 hr and 6-24 hr at low levels (<0.7% of applied dose) for groups B and C, while no parent compound was detected in dose group D. Total identified metabolites and parent compound were in the range of 78 – 83% of the applied dose for the 0-24 hr urine samples analysed by HPLC (all dose groups).

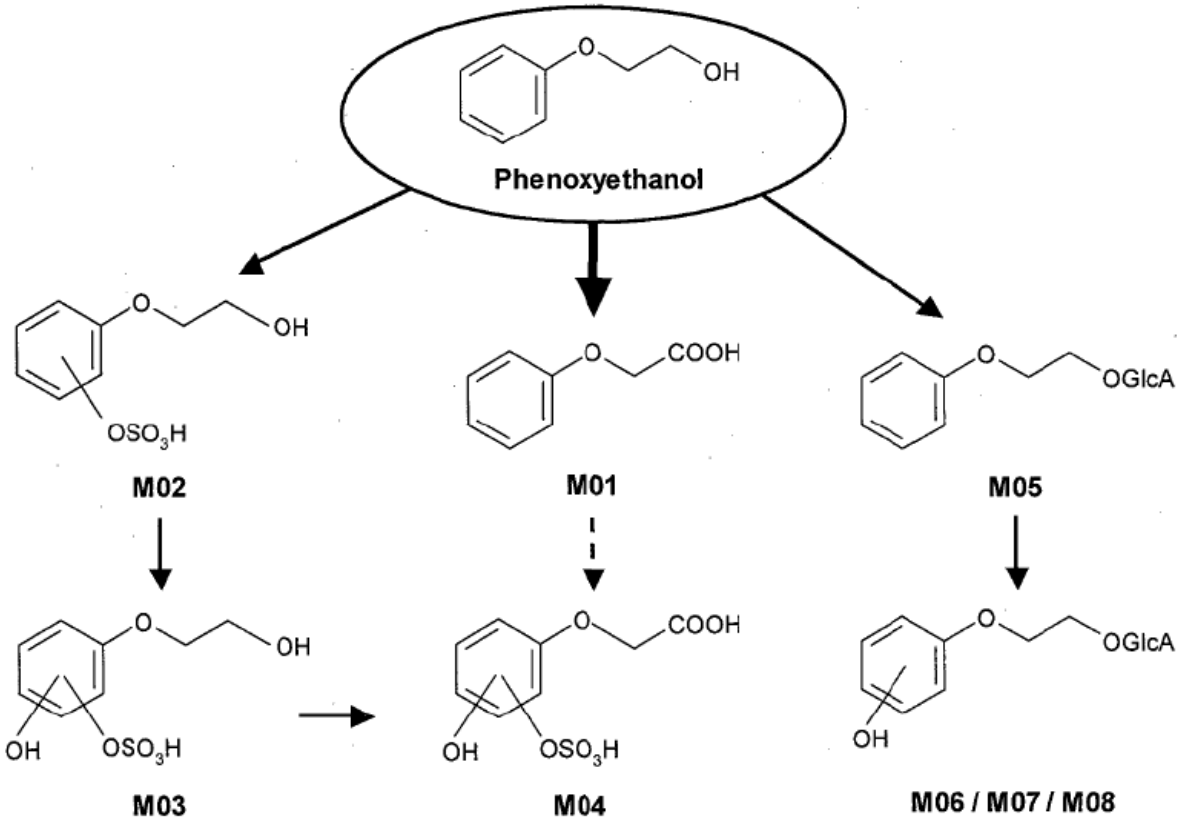
Biliary Metabolites:
Bile samples from female animals dosed with 40 mg/kg bw or 400 mg/kg bw were pooled from the sampling intervals of 0-3 hrs and 3-6 hrs and analysed by HPLC. In bile of both dose groups, only metabolites M05 (up to 2.3% of applied dose) and M01 (up to 0.4% of applied dose) were identified with M05 being the dominant metabolite. The comparison of

retention times indicated the presence of the parent compound in the pooled samples at very low levels (0.07% of the applied dose).

Faecal Metabolites:
Faeces samples were not further investigated due to very low amounts of radiolabel (1.71-2.04% of the applied dose) found in the faeces from animals in dose groups B, C, and D.

Metabolic Pathways
The metabolic pathway of 2-phenoxyethanol is depicted in **Figure 1**. The main biotransformation step is oxidation of the terminal hydroxyl group to a carboxylic acid. This yields the dominant metabolite phenoxyacetic acid. Other metabolic transformations were either ring sulfonation after hydroxylation or conjugation with glucuronic acid at the side chain, yielding the M02 or M05 metabolite, respectively. In addition, the metabolites M03, M04 (with terminal carboxyl group) and M06 – M08 (glucuronide conjugates) resulted from hydroxylation of the aromatic ring. Metabolite M04 could also be regarded as an oxidised product of metabolite M03.

Figure 1: Metabolic Pathways of 2-Phenoxyethanol in Rats



Conclusion
^{14}C-2-Phenoxyethanol was nearly completely oxidised after oral administration in the rat. In urine, less than 0.7% of the administered dose was found to be the parent compound. No parent compound was detected in plasma. 2-Phenoxyethanol was mainly metabolised to phenoxyacetic acid (PAA) by oxidation of the terminal hydroxyl group to the carboxylic acid (up to 64% of the applied dose). Several other metabolites were found in urine at <10% of the applied dose. These metabolic changes were either ring sulfonation after hydroxylation or conjugation with glucuronic acid at the side chain. In a further step, these metabolites

were mainly hydroxylated at the ring and, in one case, the terminal hydroxyl group was oxidised to a carboxylic acid.

Ref.: 28

SCCS Comment

The study is well performed. However, the question is whether the oral rat model is relevant for dermal exposures as no 2-phenoxyethanol could be detected in plasma after 1 h (T_{max} of radioactivity) and 2 hrs due to rapid first pass metabolism in the liver at single doses up to 400 mg/kg bw. Furthermore, in urine after up to 24 hrs, only traces of 2-phenoxyethanol were found.

Absorption, distribution, elimination and kinetics of 2-Phenoxyethanol in rats after i.v. and dermal exposure

In a recently published study, an LC-ESI–MS/MS method with polarity switching for the simultaneous analysis of 2-phenoxyethanol (PE) and its major metabolite, 2-phenoxyacetic acid (PAA) was developed and applied to the toxicokinetic analysis of both compounds in rat plasma, urine, and 7 different tissues.

Intravenous injection study:

Male Sprague Dawley rats (8 weeks, body weight 230–280 g) were dosed after overnight fasting by intraperitoneal application with 0.2, 0.5 and 2mg/kg (n = 8 per dose). Venous blood samples (approximately 0.2 ml) were collected from the jugular vein at 0, 2, 5, 10, 15, 30, 45 min, and 1, 1.5, 2, 2.5, 3, and 4 h after injection. Plasma samples were harvested by centrifugation at 4000 g for 10 min and stored at -20°C until analysis. Urine samples were collected for 24 h after intravenous injection.

Topical application study:

Two reference sunscreen formulations of emulsion and lotion were prepared to examine the percutaneous absorption of PE. The percutaneous absorption study was conducted in rats in accordance with the OECD TG 428 (2004). The applied amount of each formulation was 234 mg/kg, and the applied PE dose was 2.34 mg/kg (probably non-occluded conditions). Assuming an average body weight of the animals of about 250 g, around 37 µg/cm^2 were applied. Twelve hours later, the sunscreen formulations were removed from the skin. Blood samples (0.2 ml each) were collected from the jugular vein at 0, 5, 15, 30, 45 min, and 1, 1.5, 2, 3, 4, 6, 8, and 12 h after topical application. Plasma samples were harvested by centrifugation at 4000 g for 10 min and stored at –20°C until analysis.

Tissue distribution study:

The study was conducted in rats (n = 5) after constant rate intravenous infusion to steady-state. After fasting overnight, the rats were given continuous intravenous infusions for 2 h at a rate of 0.83 mg/kg/h. The infusion rate was determined as the product of the target steady state plasma concentration (C_{ss} = 100 ng/mL) and the systemic clearance obtained from the intravenous injection study. The dosing solution contained PE in isotonic saline at a concentration of 0.195 mg/mL. Blood samples were collected at 0, 15, 30, 45 min, and 1, 1.25, 1.5, 1.75, and 2 h after initiation of the intravenous infusion. Plasma samples were stored at –20°C until analysis. The animals were sacrificed after bleeding, and tissues of the brain, heart, lung, liver, spleen, kidney, and testis were collected. The steady state tissue-to-plasma concentration ratios of 2-phenoxyethanol or 2-phenoxyacetic acid were determined at 2 h after intravenous infusion and calculated as partition coefficients (Kp).

Results

The study describes a specific and sensitive LC–MS/MS assay for the simultaneous determination of PE and PAA in rat plasma, urine, and several tissues. After method validation, the assay sensitivity was adequate to quantify PE and PAA levels over a sufficient

sampling time period (e.g., >3 $t_{1/2}$) at concentrations two orders of magnitude lower than the NOAEL range. The assay achieved the LLOQ of 10 and 20 ng/mL of PE and PAA, respectively, for plasma samples and the LLOQ of 20 and 50 ng/mL of PE and PAA, respectively, for urine and tissue samples.

Intravenous injection study:
Kinetic parameter values are listed in Table X1. After intravenous injection, PE was extensively converted to PAA, with the average PAA to PE AUC ratio (AUC_{PAA}/AUC_{PE}) of 5.2, 4.5, and 5.0 for the intravenous doses of 0.2, 0.5 and 2 mg/kg, respectively. The disposition of PE was characterised by a relatively small volume of distribution (V_z, 1.6–2.0 L/kg), high systemic clearance (Cl_s, 123–132 mL/min/kg), and short terminal half-life ($t_{1/2}$, 10–11min). These values remained unaltered as a function of the injected dose range of 0.2–2 mg/kg, indicating dose-linear kinetics. Immediately after injection of PE, PAA was formed rapidly, with the time-to-peak concentration (T_{max}) of 9–10 min. For PAA, the average terminal half-life (15–34 min) and T_{max} (9 – 10 min) also remained unaltered as a function of the injected dose. PE was not found in urine, but PAA was found to be extensively excreted in urine (64.7–75.7% of the equivalent dose of PE).

Table 5 Kinetic parameters of 2-phenoxyethanol and 2-phenoxyacetic acid in rats after intravenous injection of 2-phenoxyethanol at doses of 0.2, 0.5, and 2 mg/kg (n=8 per dose, mean ± S.D.)

Parameters	Dose (mg/kg)		
	0.2	0.5	2
Phenoxyethanol			
$t_{1/2}$ (min)	9.8 ± 5.0	11.1 ± 2.5	11.4 ± 2.3
C_0 (ng/mL)	193.5 ± 40.2	464.6 ± 200.3	2177.8 ± 569.8
AUC_{inf} (ng h/mL)	32.3 ± 17.1	84.4 ± 43.1	330.0 ± 202.4
V_z (L/kg)	1.6 ± 0.9	2.0 ± 1.1	2.0 ± 0.8
Cl_s (mL/min/kg)	122.8 ± 45.2	126.2 ± 63.8	132.3 ± 63.1
Urinary excretion (%)[a]	–	–	–
Phenoxyacetic acid			
$t_{1/2}$ (min)	15.7 ± 5.1	15.4 ± 4.3	33.7 ± 29
T_{max} (min)	10.0 ± 8.9	8.7 ± 4.4	9.2 ± 9.4
C_{max} (ng/mL)	246.4 ± 142.9	597.4 ± 210.5	2280.5 ± 1052.5
AUC_{inf} (ng h/mL)	167.1 ± 155.1	377.3 ± 237.1	1638.2 ± 1436.8
Urinary excretion (%)*	75.5 ± 8.7	70.9 ± 18.3	64.7 ± 14.1

[a] Not detectable (n=4 per dose).

Topical application study:
Both PE and PAA were quantifiable in the first plasma samples (5 min) and reached C_{max} at approximately 1 h (see example, Fig. X1). PE was rapidly absorbed and, throughout the sampling period, plasma PAA levels were consistently higher than corresponding PE levels. The kinetic parameter values of PE and PAA are summarised in Table 6. The absolute topical bioavailability (F) of PE was high (mean 75.4% and 76.0% for emulsion and lotion, respectively).The apparent terminal half-life of PE found after topical application of emulsion and lotion (mean range, 96–102 min) was significantly longer than that found after intravenous injections (mean range, 10–11 min). Similarly, the apparent terminal half-life of PAA was significantly longer (108–126 min) than that found after intravenous injections

(mean range 15–34 min). The average AUC_{PAA}/AUC_{PE} ratios following topical application (mean range 4.4–5.3) were comparable to those found after intravenous injection (4.5–5.2), i.e., concentrations of free PE were around 20% of the bioavailable dose in plasma. Urinary excretion of PE and PAA was not reported.

Fig. 2 Average concentration–time profiles of 2-phenoxyethanol and phenoxyacetic acid in rats after topical application of lotion (n=6 each).

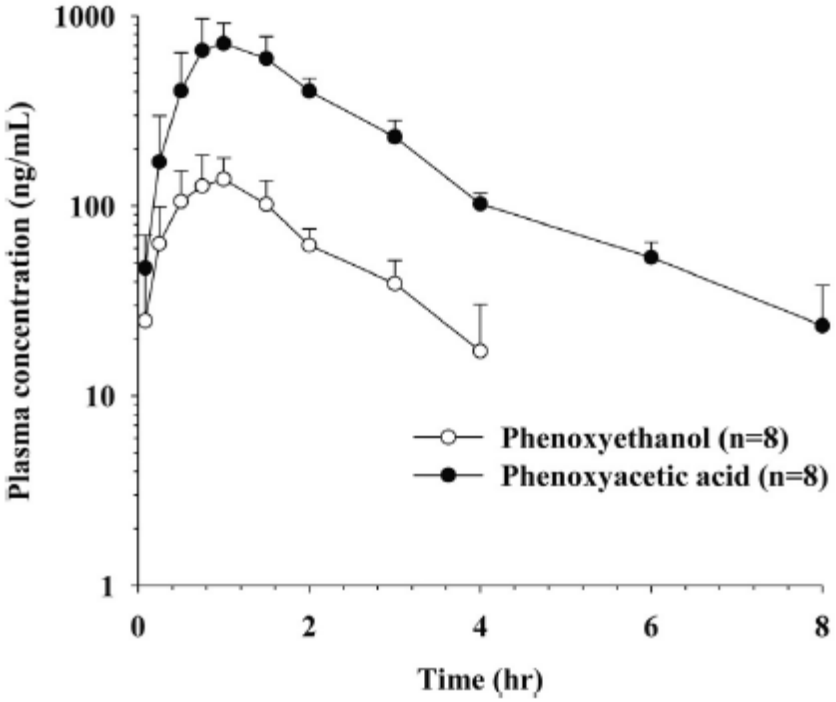


Table 6 Kinetic parameters of 2-phenoxyethanol and 2-phenoxyacetic acid in rats after topical application of emulsion or lotion containing PE at a dose of2.34 mg/kg (n=6 each, mean ± S.D.)

Parameter	Emulsion	Lotion
Phenoxyethanol		
$t_{1/2}$ (min)	96.0 ± 60.0	102.0 ± 48.0
T_{max} (min)	60.0 ± 30.0	60.0 ± 6.0
C_{max} (ng/mL)	154.4 ± 54.7	141.4 ± 46.0
AUC_{inf} (ng h/mL)	355.0 ± 188.0	334.0 ± 83.0
F (%)	75.4 ± 38.5	76.0 ± 18.1
Phenoxyacetic acid		
$t_{1/2}$ (min)	126.0 ± 48.0	108.0 ± 30.0
T_{max} (min)	72.0 ± 30.0	60.0 ± 0.0
C_{max} (ng/mL)	596.5 ± 271.6	718.9 ± 199.1
AUC_{inf} (ng h/mL)	1563.0 ± 861.0	1778.0 ± 227.0

F, bioavailability

Tissue distribution study:
After the initiation of infusion, steady-state plasma concentrations of PE and PAA were achieved within 45 min and 1.25 h, respectively. The observed steady-state plasma PE concentrations (mean113.4 ± 10.6 ng/mL) were comparable to the target concentration of 100 ng/mL. Throughout the infusion period, plasma PAA levels were consistently higher (305.0 ± 35.9 ng/mL) than corresponding PE levels. Tissue-to-plasma partition coefficients (Kp) of PE and PAA are summarised in Table X3. For PE, the highest Kp was observed for kidney (Kp = 3.9) followed by spleen, heart, brain, testis, liver, and lung with Kp values lower than 2. For PAA, the highest Kp was also found for kidney (Kp = 5.0) followed by liver (2.2) and the other organs with Kp values lower than 2.

Table 7 Tissue-to-plasma partition coefficients (Kp) of 2-phenoxyethanol and 2-phenocyacetic acid in rats (n=5, mean ± S. D.)

Matrix	Phenoxyethanol	Phenoxyacetic acid
Plasma	–[a]	–[a]
Brain	1.1 ± 0.4	–[b]
Heart	1.5 ± 0.9	0.3 ± 0.2
Lung	0.4 ± 0.1	1.2 ± 0.7
Liver	0.6 ± 0.4	2.2 ± 0.6
Spleen	1.5 ± 0.5	0.3 ± 0.1
Kidney	3.9 ± 2.0	5.0 ± 3.2
Testis	1.0 ± 0.2	1.0 ± 0.4

[a] Not available.
[b] Phenoxyacetic acid levels in brain were below the limit of quantification.

AR 9

Conclusions
In these low-dose experiments, kinetics after i.v. application were linear with doses in the range of 0.2 to 2 mg/kg bw. Kinetics after topical application were much slower than after i.v. application when comparing $t_{1/2}$ and T_{max} values of PE and PAA of both exposure routes. Rapid increase of PAA concentrations in blood after dermal application suggests some oxidative metabolism in rat skin, consistent with earlier findings *in vitro* (ref. 9; ref. 12; ref. 86). Remarkably, after both i.v. and dermal application, around 20% of the bioavailable dose appeared as free PE in blood, much more than after oral exposure. Under i.v. steady state conditions at 100 ng PE/ml in blood, the proportion of PE reached around 30% and tissue-to-plasma ratios of both PAA and PE were highest in kidney suggesting an accumulation potential in this target organ of PE or PAA toxicity and consistent with saturation of elimination at high oral doses (ref. 27).
In conclusion, portions of PE in blood after dermal exposure were found much higher than after oral exposures of rats where no PE in blood and only traces in urine could be detected due to rapid first pass metabolism in rat liver at oral doses, which were >15 to >500fold higher in the ADME studies (ref. 27 and ref. 28) than in this kinetic study. Hence it cannot be excluded that haematotoxic effects may be observed in rats after dermal exposure at 2-phenoxyethanol doses of some hundred mg/kg bw/day.

3.3.9.3 Toxicokinetics in humans

Howes (1988; 1989) reported studies on the fate of 2-phenoxyethanol in human volunteers after dermal and oral application.
An adult male volunteer ingested a dose of 10.3 mg non-labelled 2-phenoxyethanol. Urine was collected 24 hrs before dosing and for 72 hrs thereafter. 2-Phenoxyethanol was rapidly and completely absorbed and excreted in urine. Free 2-phenoxyethanol and 2-phenoxyacetic acid was extracted from urinary samples: the remaining aqueous phase was acidified and hydrolysed. Only free 2-phenoxyacetic acid (85%) and its conjugates (15%) were found by use of gas/liquid chromatography. No free 2-phenoxyethanol or its conjugates could be detected. The limit of detection of free PE was about 0.5 ppm. Calculated recovery was 104%.
In a clinical study, four hospitalised volunteers (1 male, 3 females) with skin complaints were treated with up to 40 g/day of aqueous skin cream containing 1.2% 2-phenoxyethanol, i.e. up to 480 mg/day for up to 2 days (around 7-8 mg/kg bw/day). All urine produced was collected as 24 h samples and analysed for 2-phenoxyethanol, 2-phenoxyacetic acid and any conjugates derived. From the male volunteer, urine was collected for the first 24 h as 6 h samples and subsequently as 24 h samples. Urinary excretion was rapid. The male volunteer excreted 18% of the recovered urinary amount within 6 hrs and 27% within 12 h. Only free and total 2-phenoxyacetic acid could be detected. The limit of detection of free and total 2-phenoxyethanol was 1 mg/day. Recoveries in urine up to 3 days after treatment varied from 8.5% to 42% of the dose applied.
In conclusion, in human volunteers orally exposed once to around 0.14 mg/kg bw or topically treated with up to 7-8 mg/kg bw day, only free 2-phenoxyacetic acid and its conjugates were found in urine.

Subm II, ref. 6 and 7

3.3.9.4 Physiologically based pharmacokinetic (PBPK) modelling

Troutman et al. (2015) developed a physiologically-based pharmacokinetic (PBPK) model for 2-phenoxyethanol (PE) and its metabolite 2-phenoxyacetic acid (PAA) following oral and dermal exposures to PhE in rodents and humans. The PBPK model is described in detail in **Annex 1**. The model incorporates chemical- and species-specific descriptions of physiological tissues and kinetic processes describing the absorption, distribution, metabolism and excretion of PE and PAA for oral and dermal dosimetry prediction in rats and humans. The model was verified against experimental blood, tissue and urine data across dose ranges in a species (rat) that is considered relevant to human health risk assessment. Simulations of repeated dose rat studies facilitated the selection of systemic AUC as the appropriate dose metric for evaluating internal exposures to PE in rats and humans.

The availability of human biomonitoring data in adult humans and urinary excretion of PAA in preterm infants (23-26 week gestational age) resulted in dosimetry predictions and support refinement of the default interspecies toxicokinetic uncertainty factor from 4 to 1. Therefore, the use of an overall margin of safety (MoS) value of 25 rather than the default value of 100 can be considered acceptable. Based on conservative aggregate product use assumptions, model-predicted internal exposures to PE associated with oral care and cosmetic product use in adult humans and baby care product use in infants were well below the internal exposures to PE in rats at the NOAEL dose. Calculated MoS values for PE for aggregate cosmetic use scenarios previously described in Submission I ranged from 39-359 (Table 8; see also Annex 1). For all exposure scenarios, calculated MoS values were well above the PBPK-refined margin of safety value of 25, supporting the safe use of PhE-containing products at a concentration up to 1% in both adults and babies.

Table 8 Margin of Safety (MoS) calculations for aggregate 2-phenoxyethanol exposures to adults and infants/children of 8 kg body weight

		External dose		AUC (mg*h/L)		MoS	
Sub/population	Description	mg/kg/day	BW (kg)	PhE	PhAA	PhE	PhAA
Rat	NOAEL (drinking water)	369	0.25	61.5	690	-	-
Human (adult)	Aggregate Cosmetics	2.69	60	0.608	8.82	101	78
Human (infant)	Daily Baby Care (Part 1) a	1.72	8	0.279	3.19	220	217
Human (infant)	Part 2a (Leave-on/nappy)	2.64	8	1.04	12.0	59	58
Human (infant)	Part 2b (wipes/nappy)	2.00	8	0.97	11.1	63	62
Human (infant)	Parts 1+2a+2b	6.36	8	2.29	26.3	27	26

Subm II, ref. 12

SCCS comment
The PBPK model was mainly developed by use of oral rat kinetic data. Whereas the human data described in sections 3.3.9.3 and 3.3.11 were also used, the model is primarily focused on the formation of 2-phenoxyacetic acid and kidney toxicity probably caused by this metabolite. However, based on the calculated AUC ratios of 2-phenoxyethanol and 2-phenoxacetic acid in blood after dermal exposure of rats, the model only predicts a portion of around 6% of free 2-phenoxyethanol in blood whereas a recent toxicokinetic study demonstrated a bioavailable portion of 20% and more of the parent compound (Kim et al., 2015; AR 9). Hence, the model underestimates about threefold the portion of free 2-phenoxyethanol in rats after dermal exposure (see below Tables 9-11).

Supplementary PBPK data provided in May 2016

During the commenting period of the first version of the Opinion, the applicant provided additional PBPK data taking into account the data of the study of Kim et al. (2015; AR 9), which were not yet available at the time point when the applicant provided the second submission.

To evaluate further the performance of the published PBPK model, intravenous bolus and dermal exposure simulations were performed based on descriptions of the pharmacokinetic studies published by Kim et al (2015). Since the systemic area under the plasma concentration-time (AUC) curve was selected as an appropriate dose metric in the PBPK model-based risk assessment, model-predicted AUCs were compared to those reported by Kim et al (2015) to evaluate the accuracy and reliability of the model predictions. An assessment of accuracy was determined for AUC estimations based on fold error calculations which were determined by taking the ratio of the predicted and observed values. The following results from the physiologically-based pharmacokinetic (PBPK) model were obtained, which focused on internal exposures to both 2-phenoxyethanol (PhE) and the metabolite phenoxyacetic acid (PhAA) in rats. These data were used for comparison with the experimental data.

1) Comparison of the PBPK model data with the experimental systemic exposure of 2-phenoxyethanol and 2-phenoxyacetic acid in rats after iv bolus injection of 2-phenoxyethanol at doses of 0.2, 0.5 and 2 mg/kg:

Model-predicted AUCs following i.v. bolus injection of 2-phenoxyethanol to male rats were higher by 2.7- to 2.9-fold (2-phenoxyethanol) and 1.9- to 2-fold (phenoxyacetic acid), in comparison to experimentally determined values (see Table 9).

2) Comparison of the PBPK model data with the systemic exposure of 2-phenoxyethanol and phenoxyacetic acid in rats after dermal application of sunscreen formulations containing 2-phenoxyethanol at 2.34 mg/kg (37 µg/cm2):

As shown in Figure 2, model-predicted AUCs from the published dermal PBPK model were 3.1-fold (2-phenoxyethanol) and 2.2-fold (phenoxyacetic acid) higher than the experimentally determined values, affording a conservative estimate of systemic exposure.

3) Comparison of the PBPK model data with the fraction of the systemically absorbed dermal dose of 2-phenoxyethanol in rats after dermal exposures:

As shown in Table 2 and Figure 3, following dermal exposure, the fraction of 2-phenoxyethanol in blood reported by Kim et al 2015 ranged from 0.16 to 0.19. The predicted value from the published PBPK model was 0.23.

4) Comparison of the PBPK model data with the dermal absorption data described by Vincent and Marty (2002; ref. 7) using an in vitro absorption study in human skin:

Data in figure 4 indicate that the predicted biomarker levels average within 94% of the measured values thus confirming the accuracy of the model to predict the in vitro dermal penetration of 2-phenoxyethanol through human skin following dermal dosing of 0.2% and 1.0% 2-phenoxyethanol concentrations in a cleaning gel rinse-off formulation and a leave-on body lotion formulation.

Table 9: Summary of predicted and experimental plasma AUC values in rats following intravenous bolus dosing of 2-phenoxyethanol at 0.2, 0.5 and 2.0 mg/kg. AUC values are reported in units of mg*h/L

	PBPK Prediction	Experimental		Fold error	AUC ratio (PhE/(PhE+PhAA)	
Dose level	AUC	Mean AUC	St. Dev	(Pred/Exp)	Predicted	Experimental
(mg/kg)	2-phenoxyethanol					
0.2	0.0921	0.0323	0.0171	2.9	0.232	0.162
0.5	0.230	0.0844	0.0431	2.7	0.232	0.183
2.0	0.926	0.330	0.2024	2.8	0.231	0.168
	2-phenoxyacetic acid					
0.2	0.305	0.1671	0.1551	1.8	-	-
0.5	0.764	0.3773	0.2371	2.0	-	-
2.0	3.08	1.6382	1.4368	1.9	-	-

Table 10: Summary of predicted and experimental plasma AUC values in rats following dermal exposures in two sunscreen formulations at 2.34 mg/kg (37 ug/cm²). AUC values are reported in units of mg*h/L

	2-phenoxyethanol AUC			2-phenoxyacetic acid AUC			
	Mean	St. Dev	Fold error (Pred/Exp)	Mean	St. Dev	Fold error (Pred/Exp)	AUC ratio (PhE/(PhE+PhAA)
PBPK Prediction	1.08	-	3.1 [a)]	3.60	-	2.2 [a]	0.231
Experimental (emulsion)	0.355	0.188	-	1.563	0.861	-	0.185
Experimental (lotion)	0.334	0.083	-	1.778	0.227	-	0.158

[a)] = -fold error was calculated using the average AUC reported for the emulsion and lotion formulations

Table 11: Summary of experimental and PBPK model-predicted dose metric values across species, dose levels and dose routes

				AUC (mg*h/L)			AUC (mmol*h/L)		Mass AUC ratio:	Molar AUC ratio:
Species	Dose Route	Dose (mg/kg)	Data Source	PhE	PhAA	PhE+PhAA	PhE	PhAA	PhE/ PhE+PhAA	PhE/ PhE+PhAA
Rat	iv bolus	2.0	Kim et al 2015	0.330	1.64	1.97	0.00239	0.0108	0.168	0.182
Rat	oral bolus	152	Louisse et al 2010	35.6	271	307	0.258	1.7812	0.116	0.126
Rat	dermal (lotion)	2.34	Kim et al 2015	0.334	1.78	2.11	0.00242	0.0117	0.158	0.171
Rat	dermal (emulsion)	2.34	Kim et al 2015	0.355	1.56	1.92	0.00257	0.0103	0.185	0.200
Rat	mean dermal	2.34	Kim et al 2015	0.345	1.67	2.02	0.00249	0.0110	0.171	0.185
Rat	iv bolus	2.0	PBPK	0.926	3.08	4.01	0.00670	0.0203	0.231	0.249
Rat	oral bolus	152	PBPK	31.4	344	376	0.227	2.26	0.0835	0.0912
Rat	dermal	2.34	PBPK	1.08	3.60	4.68	0.00783	0.0236	0.231	0.249
Human	dermal	2.34	PBPK	1.82 [a)]	19.1	20.9	0.0131	0.125	0.087	0.0949

[a)] For the aggregate daily dose of 2.69 mg/kg/d in adult humans, the following values result: AUC_{PhE} = 2.09 and AUC_{PhAA} = 22.0 mg*h/L, respectively.

SCCS comment

The updated PBPK data for dose metric AUC of phenoxyethanol (AUC_{PhE}) in adult humans (last line of table 11) are around 3-fold higher than those predicted by Troutman et al. (2015, subm II, ref. 12; see Table 8) resulting in MoS values of around 30 for adults when comparing AUC_{PhE} in human adults with the respective AUC value in rats at the NOAEL (Table 8). This considerable increase of internal exposure of adults to phenoxyethanol (and also phenoxyacetic acid) by dermal exposure was not commented by the applicant. No

supplementing data were provided for infants/children exposure groups, for which the resulting MoS values may be around 10-15.No data on C_{max} as a dose metric have been provided by the applicant. It can be assumed that C_{max} is not a relevant dose metric due to slow dermal absorption of 2-phenoxyethanol.
The SCCS agrees that the rat PBPK model essentially fulfills the WHO's recommendations (WHO 2010, AR 8): The rat PBPK model can reproduce the chemical-specific pharmacokinetic data under various experimental conditions for oral route and dermal application and is reliable with regard to its predictions of dose metrics for 2-phenoxyethanol (PhE) and phenoxyacetic acid (PhAA) (Cmax AUC, renal clearance).

Regarding the human PBPK model, the PBPK model can reproduce the chemical-specific pharmacokinetic data under two experimental conditions for oral route and dermal application and is reliable with regard to its predictions of only one dose metrics: cumulative PhAA in urine.

The human data (cumulative urine excretion of PhAA) were used for calibration of the human PBPK model. Unfortunately, a comparison between AUC (PhE or PhAA) observed and AUC (PhE or PhAA) predicted was not performed, which limits the applicability of the PBPK modeling for humans.

The SCCS considers that the human PBPK model cannot be used for the current evaluation for two reasons:

- The dose metric considered for the Margin of Safety (MOS) calculations is AUC, whereas no measured data in human blood is available. Consequently, no validation for the human PBPK model concerning the selected dose metric (AUC) is available. The confidence in the human PBPK model is considered low for this relevant (AUC) internal dose metric.

- The human data (cumulative urine excretion of PhAA) were used for calibration of the human PBPK data. The lack of human data for validation (comparison between observed and predicted for cumulative excretion) limits the applicability of the PBPK modeling for humans.

In the biomonitoring studies used by Troutman et al. (2015), the dose levels were unspecified, i.e. external exposure was estimated but not measured (Göen et al., 2001, ref. 30; Fromme et al., 2013, subm II, ref. 11; and Garlantezec et al., 2012, ref. AR 13). SCCS considers that the human PBPK model (once validated) could be used to estimate a corresponding external exposure from measured PhAA concentrations in urine, but not to verify the model.

Under these considerations, additional model evaluation would be needed regarding the reliability (model testing, uncertainty and sensitivity) as described and recommended by ref. AR 8 (WHO 2010) in figure 10.

Additional information is required regarding the relationship between blood concentration, AUC and renal clearance in the human model.

3.3.10 Photo-induced toxicity

No data available.

3.3.11 Human data

Human toxicokinetic data see 3.3.9.3.

Studies from clinical exposures

Gough et al. (1944) reported on the use of a 2.2% (v/v) aqueous solution of 2-phenoxyethanol for the treatment of superficial wounds that were infected by bacteria. Treatment for up to several weeks in 8 cases was successful and no adverse effects were reported. The maximum amount used per day were 40 ml of the solution corresponding to around 1 g 2-phenoxyethanol per day.

Subm II, ref. 8

In another clinical study, 23 cases with burns (<15% of the body surface) were treated with 2 % 2-phenoxyethanol in a cream (ca. 1.4 mg 2-phenoxyethanol/cm^2, maximum duration 4 days). Assuming 15% body surface (2625 cm^2) and a body weight of 70 kg, the daily maximum amount applied was about 50 mg/kg bw. No adverse effects during this trial were reported. However, no special investigations on potential neurotoxic signs were conducted (Lawrence et al., 1982; reported by DFG 1998).

Subm II, ref. 9; AR 10

Morton (1990) reported on 2-phenoxyethanol exposure of 3 women that were employed in a fish hatchery. During the season from February to September, they had to tag young fish and to trim their fins in dishpans filled with water. Small amounts of 2-phenoxyethanol were added to the water to anaesthetise the fish. The concentration was not reported. Each tagger used about one 500 ml bottle of 2-phenoxyethanol and bare-handedly tagged or trimmed some about 4000 to 8000 fish per day. The principal route of exposure was dermal absorption, but some degree by inhalation is also possible. When using phenoxyethanol, they generally noted headache, lightheadedness, slurred speech, euphoria, grogginess and eventually feeling "drunk". After some hours of work, there would be diminished strength and sensation of hands, particularly in the hand used to pick up the fish. Phenoxyethanol in blood or urine was not determined. After 1-2 years of this kind of exposure, the workers experienced onset of constant irritability, forgetfulness and difficulty maintaining concentration. Neuropsychological test conducted several months or some years after exposure confirmed persistent impairments of cognitive functions such as learning and also dexterity. However, no details of testing were reported. As other factors such as predisposition could not be ruled out, the extent of occupationally acquired deficits by this kind of exposure remain unclear.

Subm II, ref. 10

Study in Premature Neonatal Infants

Bührer et al. (2002) investigated the skin tolerability of an aqueous solution containing 0.1% octenidine and 2% 2-phenoxyethanol for skin disinfection during the first 7 days of life in premature neonates of <27 weeks gestation (N=24). In boys (N=13), spot urine was sampled at 4 h intervals and analysed for the presence of 2-phenoxyethanol and its metabolite, 2-phenoxyacetic acid by high-pressure liquid chromatography. In the most immature neonate (gestational age 23 6/7 weeks), a transient erythematous reaction was observed following application of the octenidine/phenoxyethanol solution prior to umbilical vessel catheterisation. No other local reactions were observed. The urinary concentration of 2-phenoxyethanol was <2 ppm in all samples, while urinary 2-phenoxyacetic acid concentrations reached 5-95 ppm (median 24 ppm).
These results indicate that 2-phenoxyethanol at a concentration of 2% was well tolerated when applied to the skin of premature neonates and that 2-phenoxyethanol, while readily

absorbed by the neonate's skin, undergoes extensive oxidative metabolism to 2-phenoxyacetic acid.

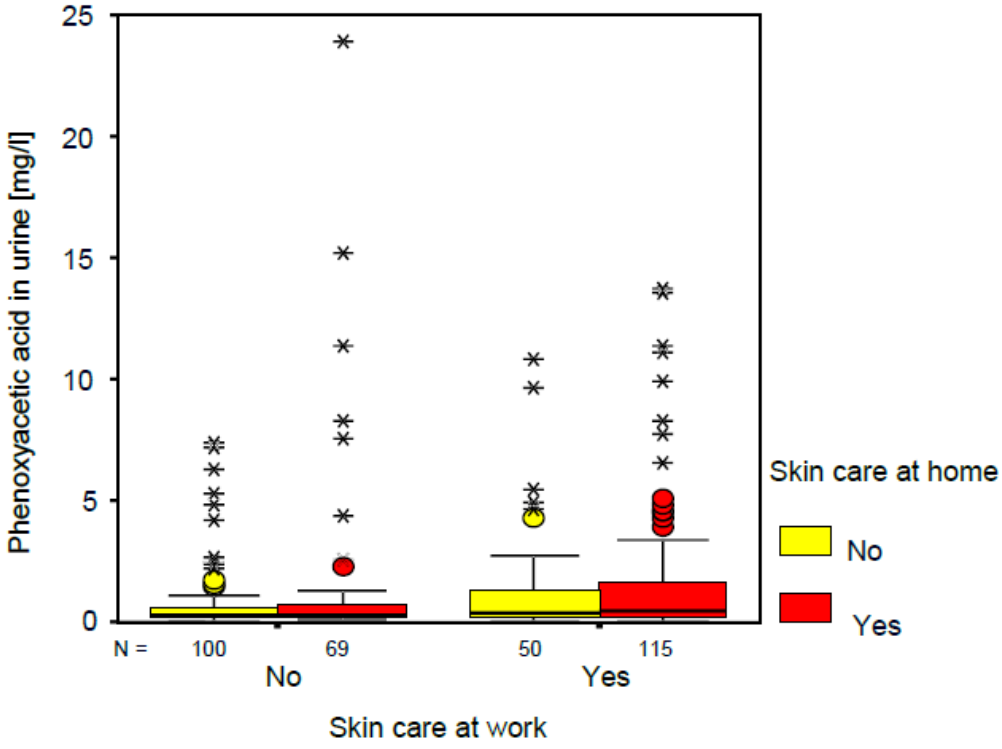
Ref.: 29

Human Biomonitoring Studies

In a study conducted by Göen et al. (2001), urine samples were taken from a cohort of 379 employees of the German printing industry who were not occupationally exposed to 2-phenoxyethanol. The cohort consisted of 237 printers (Group A) and 142 individuals employed in packing halls (Group B). The individuals were 20 – 63 years old and the majority were males (8% females). The urine samples were analysed for 2-phenoxyacetic acid, the major metabolite of 2-phenoxyethanol. 2-Phenoxyacetic acid was detected (≥0.1 mg/l) in 89% of the samples.
The range was <0.1 – 151.0 mg/l, the median was 0.3 mg/l and the 95th percentile value was 6.3 mg/l. For Group A, who reported using skin care products at work with a larger distribution than Group B, the group median was 0.4 mg/l and the 95th percentile value was 7.8 mg/l. For Group B, the group median was 0.2 mg/l and the 95th percentile was 4.9 mg/l. **Figure 3** shows the distribution of 2-phenoxyacetic acid concentration in urine among 334 members of the cohort who were asked on whether they used skin care products at home, at work, both at home and at work, or neither at home or at work. Skin care product use at work had the most influence on the median urinary 2-phenoxyacetic acid concentration, although a few individuals who reported using skin care products only at home had higher urinary 2-phenoxyacetic acid concentrations, with the highest value in this group being around 24 mg/l.

Ref. 30

Figure 3
Use of skin care products by workers and systemic exposure to 2-phenoxyethanol



Data from Göen et al. (2001), ref. 30

Fromme et al. (2013) analysed the background levels of 11 glycol ether metabolites in Southern Germany in 2007/2008. 24-h urine samples were collected from 31 female and 13 male habitants who were not exposed to glycol ethers occupationally. The age of the

participants ranged from 19 to 52 years. 2-Phenoxyacetic acid (PAA) could be detected in each urine sample with a median value of 0.80 mg/L, a 95th percentile of 23.6 mg/L, and a range of 0.01–47.4 mg/L, respectively. Whereas for most of the glycol ether metabolites very low levels were found and 95th percentiles did not exceed 0.3 mg/L, levels of PAA in urine were much higher and significantly higher values were determined in the urine of female participants than in that of male test persons (means: 5.9 mg/L versus. 1.2 mg/L). An explanation for the high urinary excretion levels of PAA may result from the use of 2-phenoxyethanol as a preservative in numerous personal cleansing, cosmetic and skin care products. This explanation complies also very well with the findings of higher PAA levels in females. Compared with earlier levels of PAA in the German population (Göen et al., 2001, group B), the 95th percentiles were lower (4.9 mg/L; N = 142) at that time than in the recent study.

Subm II, ref. 11

In other studies, varying results in background studies were reported (overview by Fromme et al (2013), subm II, ref. 11).

Table 12 Background levels of 2-phenoxyacetic acid in urine samples (mg/L)

Reference	N	Median	95-Percentile	Range	Comments
Multigner et al. (2007), AR 11	53	~<0.05 [a)]		~<0.05 – 2.03 [a)]	France 2000/01
Labat et al. (2008), AR 12	200	0.58 [a)]		<LOD - 73.9 [a)]	France
Fromme et al. 2013, Subm II, ref. 11	44	1.33 [a)]	28.3 [a)]	0.02 – 79.1 [a)]	Germany 2007/08
Garlantézec et al (2012), AR 13	451	0.49 [b)]		<0.05 – 36.0 [b)]	France 2002 – 2005
Fromme et al. 2013, Subm II, ref. 11	44	0.8 [b)]	23.6 [b)]	0.01 – 47.4 [b)]	Germany 2007/08

LOD, Limit of detection
[a)] Values in mg/g creatinine
[b)] Values in mg/L

Subm II, ref. 11

In conclusion, most median values of biomonitoring studies were in the range of 1 mg/L (or mg/g creatinine), while values in the upper range were more than 20-fold higher.

Clinical Evaluation of Objective and Sensory Skin Irritation

Lee et al. (2007) investigated the objective and subjective skin irritation potential of various preservatives used in cosmetics, including 2-phenoxyethanol. In the first part of the study, solutions of various preservatives were prepared for 24 hr occlusive patch testing in 20% aqueous ethanol at concentrations 5 times the minimal inhibitory concentration (MIC) for *Pseudomonas aeruginosa*. For 2-phenoxyethanol the MIC was 0.4%, so the test concentration was 2%. No difference in mean score (n=31 subjects) was observed for 2% 2-phenoxyethanol (0.16+0.45) as compared to the vehicle (0.10+0.55). Sensory irritation in lactic acid sensitive subjects was evaluated with the preservatives tested at the MIC. A mean score sensory irritation score of 0.34 (using a scale of 0 – 3) was observed for 0.4% 2-phenoxyethanol.

In the second part of study, the preservatives were formulated in four different types of emulsion bases intended to be representative of cosmetic formulations. Combinations of preservatives were used in all four formulations and 2-phenoxyethanol was used in two of these formulations at a concentration of 0.3%, along with two or three other preservatives. These formulations were tested in a 21-day cumulative irritation test and in a sensory irritation test using lactic acid sensitive subjects. All formulations showed weak responses in the 21-day cumulative irritation test, and there was no statistical difference in responses between different combinations of preservatives. Sensory irritation varied by formulation type, but the presence of 0.3% 2-phenoxyethanol as part of a combination of preservatives had little or no influence on sensory irritation scores.

Ref.: 31

SCCS comment

No conclusion on the skin irritation potential of 2-phenoxyethanol at a concentration of 1% can be drawn from this kind of study.

Human Studies on the Prevalence of Sensitisation

The frequency of sensitisation to preservatives used in cosmetic products, including 2-phenoxyethanol, has been the topic of investigation of several studies in patients with suspected allergic contact dermatitis. Data from the IVDK, a contact allergy surveillance network in Germany, Switzerland, and Austria, were used to determine the frequency of sensitisation to 2-phenoxyethanol among a total patch-test population of 6932 evaluated through this network. The percentage of patients with positive patch test results to 1% 2-phenoxyethanol (standardised for sex and age) was 0.24% (ref. 32). The risk of sensitisation to preservatives has also been evaluated by taking into account the prevalence of use of the various preservatives across a set of 3541 leave-on products, based on product labelling information. In this study, 1111 products were reported to contain 2-phenoxyethanol. Among 4995 patients patch tested, the frequency of positive responses to 2-phenoxyethanol in patients was 0.14%. An index referred to as the sensitisation exposure quotient (SEQ), calculated as the quotient of the relative frequency of sensitisation and the relative frequency of use, was used to rank the various preservatives evaluated. The SEQ for 2-phenoxyethanol was 0.06, the lowest value among all the preservatives evaluated, and it was concluded that the risk of becoming sensitised to this preservative is clearly negligible (ref. 33).

In an earlier study utilising the IVDK network data from 1 January 1990 to 31 December 1994, among 11,120 patients patch tested with 1% 2-phenoxyethanol, the frequency of positive responses was 0.1% (ref. 35) Another smaller study conducted in Spain also reported a low frequency of patch test positive results in dermatology clinic patients. In this study of 419 male and 673 female patients, the frequency of patch test positives to 1% 2-phenoxyethanol was 0.2% (ref. 34).

Ref.:32, 33, 34, and 35

Other studies in clinical (patch-tested) populations also reported low frequencies of positive reactions to 2-phenoxyethanol; rates vary from zero to 0.2% (reviewed in Scognamiglio et al, 2012, ref 105) (ref. 34, 71, 79, 90, 95, 109, 111, 116). In addition, there are a few case reports on contact urticaria (ref. 29, 63 and 64).

3.3.12 Special investigations

Mechanistic Studies – Haemolysis in Various Species *in vitro*

Guideline:	No pertinent OECD guideline for this investigative study
Species/strain:	Rabbit, mouse, rat, dog, and human (female) red blood cells
Replicates:	1-3 animals/subjects per trial

Test substance:	2-Phenoxyethanol (2-PE)
Batch No.:	41183068EO
Purity:	≥99.9%
Concentrations:	First trial: 0.938, 1.875, 3.75, 7.5, 15, and 20 mg/ml;
Second trial:	5.0, 7.5, 10.0, 12.5, and 15 mg/ml
Vehicle:	Phosphate buffered saline
Reference Controls:	2-Phenoxyacetic acid, 2-Ethoxyethanol (2-EE), 2-Ethoxyacetic acid (2-EAA)
GLP:	No
Report Date:	2007

To improve the human risk assessment for 2-phenoxyethanol (2-PE), the sensitivity of red blood cells (RBC) from mice, rats, rabbits, dogs and human to *in vitro* haemolysis by 2-PE was determined. Conversion of 2-PE to 2-phenoxyacetic acid (2-PAA) occurs rapidly *in vivo* and is considered to be an inactivation pathway for the haemolytic activity of 2-PE. Therefore, a comparison of the *in vitro* potency of the parental chemical and this metabolite was performed. 2-Ethoxyethanol (2-EE) and 2-ethoxyacetic acid (2-EAA) were also chosen for comparison. 2-EE has also been shown to cause haemolysis in rats though probably to a lesser degree than 2-PE.

The resistance of the RBC to lysis was determined with various concentrations of test compounds at different time intervals. The method employed is based on the integrity of the RBC membrane and determines the degree of cell damage and the resulting haemolysis after agitation of the cell suspensions at different test compound concentrations and time intervals. After damage of the RBC membrane by the test procedure, haemoglobin is released into the test solution. The concentration of released haemoglobin is measured, and correlated directly with damage caused to the RBC membrane by the test material.

Stock solutions of the respective test compounds were prepared in phosphate buffered saline (PBS). After pH-adjustment, they were mixed in a ratio of 3 volumes of test solution to 1 volume of RBC preparation to yield final test concentrations from 20 mg/ml to 0.938 mg/ml. The test mixtures were incubated at room temperature with agitation for 0.5, 1, 2, and 4 hours, respectively. After incubation and centrifugation of the samples, the release of haemoglobin into the supernatant was determined by spectrophotometry at a wavelength of 540 nm. Results were compared to a sample totally lysed with distilled water (100% lysis) as the positive control and to a fragility control with PBS (spontaneous, not substance-related haemolysis (base line)). All samples were evaluated in triplicate.

Calculation of percentage haemolysis:
The percentage haemolysis produced by each test solution was calculated for each sample as follows:
The measured absorbance (A) of test solution (Test) and positive control (PC = 100 % haemolysis) were adjusted (adj.) against absorbance (A) of the fragility control (FC):

A(adj. Test) = A(Test) –A(FC)
A(adj. PC) = A(PC) – A(FC)
The adjusted absorbance of the test solution (A(adj. Test)) was then compared to the absorbance of the positive control (A(PC)) to give the percentage haemolysis (H).
H(%) = A(adj. Test) / A(adj. PC) × 100

Results
Total haemolysis caused by 2-PE was observed in a concentration range of 12.5 to 10.0 mg/mL in Red Blood Cell (RBC) suspensions of the different species investigated. The results of the haemolysis tests showed the following relative resistance to lysis from greatest to least: human > dog > rat ≈ rabbit > mouse. Human RBCs were, therefore, more resistant to 2-PE than RBCs of rabbit, dog, rat, and mouse. 2-PAA, 2-EE and 2-EAA

did not show significant haemolytic effects at any concentration in any of the species examined.

Conclusion
A comparison of *in vitro* 2-phenoxyethanol-induced haemolysis of red blood cells (RBC) from humans, rats, rabbits, mice, and dogs by 2-phenoxyethanol indicates that human RBCs are more resistant to lysis than RBCs from these other species.

Ref.:12 and 36

Haemolytic investigations in rabbits and rats

Guideline:	No pertinent OECD guideline for this investigative study
Species/strain:	Rabbit/New Zealand White and Rat/Fischer 344
Group size:	**Parts 1, 2A and 2B**: 3 female rabbits/dose group, **Part 3**: 3 female rats/dose group
Test substance:	2-Phenoxyethanol
Batch:	53 (C44172)
Purity:	99.9%
Dose levels:	Parts 1 and 2A: 0, 100, 300, 600 and 1000 mg/kg bw/day; Part 2B: 800 mg/kg bw; Part 3: 0, 1250 and 2500 mg/kg bw/day
Vehicle:	None (undiluted test substance)
Route:	Oral, gavage
Exposure period:	Part 1: Daily dosing until a haemolytic response was observed or animals became moribund; Part 2A: 10 days; Part 2B, single dose with blood samples taken at 1, 3, 6, and 24 hrs; Part 3: 14 days
GLP:	Yes
Report Date:	1986

Study design and methods
This study was conducted to characterise the haemolytic response in rabbits and to evaluate differences in species susceptibility between rabbits and rats.
The objective of **Part 1** of the study was to evaluate oral exposure in rabbits for reliability and consistency in inducing a haemolytic response.
In **Part 2**, the 2-phenoxyethanol-induced haemolytic response in rabbits was characterised to better understand the nature of response.
In **Part 3**, the susceptibility of the rat was evaluated.
In **Part 4**, an *in vitro* evaluation of the haemolytic dose response in rabbit red blood cells to 2-phenoxyethanol and its major metabolite, 2-phenoxyacetic acid was conducted.
The dosing schedules and exposure duration for the various parts of the study were as described above.

In **Part 2A**, blood samples for haematological evaluation were taken at necropsy or at relevant time points in relation to development of the haemolytic episode. Urine samples were taken for urinalysis and selected tissues were taken at necropsy for histopathological examination.
In **Part 2B**, blood samples were taken at 1, 3, 6, and 24 hrs after exposure for haematological evaluation and for measurement of serum levels of 2-phenoxyethanol and 2-phenoxyacetic acid. Urine was collected at necropsy, and selected tissues were taken at necropsy for histopathological examination.
In **Part 3**, blood samples were taken for packed cell volume determination, and gross pathology was evaluated at necropsy.
In **Part 4**, red blood cells from a female rabbit were incubated with 2-phenoxyethanol or 2-phenoxyacetic acid at concentrations of 0, 0.01, 0.05, 0.10, 0.50, 1.0, 1.5, and 2.0% for 0.5, 1 or 2 hrs. Aliquots were taken to determine the degree of haemolysis.

Results

Part 1

Treatment-related deaths of one rabbit/group occurred in the 300 and 1000 mg/kg bw/day groups, and the remaining two rabbits in these groups as well as the three rabbits dosed at 600 mg/kg bw/day were sacrificed moribund. All rabbits showed signs of toxicity, characterised by lethargy, respiratory distress and darkened urine that were dose-related in severity and occurrence. Mean packed cell volume (PCV) was low in blood samples taken from all moribund animals. Urine samples showed elevated levels of protein, and microscopic examination of urinary sediments revealed the presence of haemoglobin casts in some rabbits. Treatment-related microscopic changes were observed in the spleen, bone marrow, kidneys, and stomach from some animals in all treatment groups.

Part 2A

Three rabbits (one each from the 300, 600, and 1000 mg/kg bw/day groups) died during the 10-day treatment period. In addition, four rabbits (two from the 600 mg/kg bw/day group and two from the 1000 mg/kg bw/day group) were sacrificed *in extremis* prior to completion of the 10-day exposure. Average duration of exposure for the study groups was 10, 10, 10, 3.3., and 1.3 days for rabbits administered 0, 100, 300, 600, and 1000 mg/kg bw/day, respectively. Signs of toxicity were characterised by anorexia, lethargy and excretion of red-brown urine.

Rabbits exposed to 2-phenoxyethanol showed decreased red-blood cell numbers (RBC), haemoglobin (HgB), and packed cell volume. Many rabbits exhibiting severe depressions in RBC showed concurrent increases in mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets and white blood cells (WBC), although rabbits given 300 or 100 mg/kg bw/day for 10 days exhibited only slight signs of a haemolytic response.

Elevated levels of protein, bilirubin, blood, urobilinogen, WBC, RBC, epithelial cells and casts were observed in urine. Gross observations at necropsy in animals dosed with 600 and 1000 mg/kg bw/day were consistent with haemolytic anaemia, including enlarged and dark kidneys and spleen, bloody or dark coloured urine in the bladder. No gross observations were noted in rabbits dosed with 300 or 100 mg/kg bw/day. Treatment-related microscopic changes were observed in some rabbits from all dose levels and were generally related to the haematopoietic system, including slight to moderate erythroid hyperplasia in bone marrow and changes in spleen (red pulp congestion, erythrophagocytosis, and haematogenous pigment in red pulp regions). One rabbit dosed with 300 mg/kg bw/day had thrombi in venous sinuses in the spleen resulting in generalised splenic necrosis as well as thrombi within lung blood vessels. Treatment-related changes were also reported in the kidneys including haemoglobin casts in tubules and collecting ducts and degeneration and necrosis of tubule epithelium. Necrosis of stomach glandular mucosa was observed in some animals from all treatment groups.

Part 2B

Significant increases in red blood cell fragility were observed in rabbits dosed with 800 mg/kg bw when compared to controls at 1 and 3 hrs after dosing. However, no significant differences in RBC, HgB, PCV, MCH, MCHC, and platelets compared to control were observed at 1, 3, or 6 hrs post-dose. In addition, no significant differences in RBC methaemoglobin or glutathione were observed between control and treated animals during the 24-hr sampling period. At 24 hrs, two rabbits showed a sharp drop in RBC, PCV, and HgB, coupled with an increase in MCH, MCHC, and platelets. The 24-hr platelet values may have been artificially elevated due to interference from fragmented erythrocytes. Analysis of serum samples for 2-phenoxyethanol and 2-phenoxyacetic acid demonstrated that 2-phenoxyethanol was extensively metabolised to 2-phenoxyacetic acid with a peak value of 1452 ppm measured 3 hrs after dosing. Low levels of 2-phenoxyacetic acid (0-26 ppm) were detected, primarily in samples taken at 1 hr after dosing.

Part 3
Four rats (two at 1250 mg/kg bw/day and two at 2500 mg/kg bw/day) died during the course of the study and one rat receiving 2500 mg/kg bw/day was sacrificed *in extremis* prior to completion of the 14-day exposure period. The average number of doses administered to rats in the 1, 1250 and 2500 mg/kg bw/day treatment groups was 14, 6.3, and 1.0, respectively. Rats administered either 1250 or 2500 mg/kg bw/day did not exhibit clinical signs indicative of haemolytic crisis (e.g., dark or red urine) at any time during the treatment period. Signs of toxicity included lethargy and ataxia at 1250 mg/kg bw/day as well as loss of consciousness for rats dosed with 2500 mg/kg bw/day. A decreased PCV was reported in the one rat that survived 14 daily doses of 2-phenoxyethanol. Two animals administered 2500 mg/kg bw/day that died during the dosing period exhibited hyperemia of the glandular stomach mucosa upon gross pathology examination. No other specific treatment-related gross pathology was observed in this treatment group. Two rats given 1250 mg/kg bw/day that died during the dosing period exhibited erosions and haemorrhage in the stomach glandular mucosa and dark but normal-sized spleens. The one rat given 1250 mg/kg bw/day that survived to study termination showed no treatment-related lesions at gross necropsy.

Part 4
In vitro incubation of 2-phenoxyethanol with rabbit RBCs demonstrated complete lysis at concentrations of 1.0% and higher at 0.5 hr. 2-Phenoxyacetic acid did not produce significant lysis at any concentration at this time point.

Conclusions
The results of this study show that 2-phenoxyethanol causes a regenerative intravascular haemolytic anaemia in female rabbits following oral exposure. Female rats were considerably more resistant to the haemolytic effects of 2-phenoxyethanol with only minimal evidence of anaemia following repeated oral exposure to high doses. The mechanism of haemolysis does not appear to involve oxidative damage to erythrocytes, since no changes in RBC methaemoglobin or glutathione were observed.
An *in vitro* experiment with rabbit red blood cells indicates that 2-phenoxyethanol is considerably more haemolytic than its major metabolite, 2-phenoxyacetic acid (see also ref. 12, ref. 36).

Ref.: 37

3.3.13 Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Adults:		
Daily aggregate exposure for preservatives*	**A**	**= 269 mg/kg bw/day**
(default value for adults)		
Max. concentration in the final product(s)	**C**	**= 1%**
External exposure dose		**= 2.69 mg/kg bw/day**
No observed adverse effect level		
(90-day, dermal, rabbit, 500 mg/kg bw/day		
adjusted for 5 days exposure/week)	**NOAEL**	**= 357 mg/kg bw/d**

Margin of Safety, adults	**adjusted NOAEL/SED**	**= 130**

* See chapter 4-2 of the SCCS' Notes of Guidance

Given the much higher capacity of humans to metabolise 2-phenoxyethanol compared with rabbits (the most sensitive species towards 2-phenoxyethanol), the toxicokinetic default factor of 4.0 can be reduced to 1.0 yielding a minimum **MoS of 25** instead of 100 for the

safety assessment of 2-phenoxyethanol. A **MoS of 130** was derived for adult humans using an adjusted NOAEL of 357 mg/kg bw/day from a 90-day dermal RDT study in rabbits.

MoS for children
Whereas children ≤3 years may be higher exposed than adults to 2-phenoxyethanol in cosmetic products, **MoS values** of **around 50** were derived from reasonable worst case scenarios (Troutman et al. 2015, subm II, ref. 12; ANSM 2012, AR 15) (see Discussion). Therefore, the span of these MoS values of around 50 to the minimum MoS of 25 also covers the safety of newborns/infants, who may have a higher exposure to 2-phenoxyethanol exposure in cosmetic products than adults.

3.3.14 Discussion

Physico-chemical properties
2-Phenoxyethanol is soluble in water and in all common organic solvents. The vapour pressure is low to moderate. Evaporation may occur when the substance is applied on skin. Inhalation of evaporated substance is possible.

Function and uses
2-Phenoxyethanol is used as a preservative in cosmetic formulations at a maximum concentration of 1.0%.
Scognamiglio et al. (ref. 105) reported that 2-phenoxyethanol is a fragrance ingredient used in many fragrance mixtures. It may be found in cosmetics as well as in non-cosmetic products such as household cleaners and detergents. It has been reported to occur in some fruits and plants. The worldwide volume of 2-phenoxyethanl use as a fragrance ingredient in all finished consumer product categories is in the region of 100-1000 metric tons per year. The use as a fragrance ingredient in 10 types of the most frequently used personal care and cosmetic products was calculated to result in about 0.0476 mg/kg bw/day (upper level as 97.5 percentile).

Toxicological Evaluation
The toxicity profile 2-phenoxyethanol was investigated in pre-clinical studies assessing acute and repeated dose toxicity, genotoxicity, reproductive toxicity, prenatal developmental toxicity, irritation, sensitisation, percutaneous absorption, toxicokinetics and special investigations (haemotoxicity *in vitro* in various species).
Data on human exposure to 2-phenoxyethanol and data on the irritant potential and sensitisation in humans are also available.

Acute toxicity
One study from 1982 on acute oral toxicity in rats resulted in LD-50 values between 2 and 4 g/kg bw. Two other acute oral toxicity studies in rats and rabbits, which date from 1980 and are poorly documented, resulted in LD-50 values of 1- 2 g/kg bw.
In an acute dermal toxicity study with rabbits exposed to 2.2 g/ kg bw on occluded skin, no mortalities and no gross pathological findings in any of the major organs were reported. However, the kidney, a target organ of PE in rabbits, was not investigated/reported.

Local toxicity
Skin irritation:
Under the conditions of the *in vivo* study, undiluted 2-phenoxyethanol is considered as a mild irritant to the rabbit skin.

Eye irritation:
Under the conditions of the *in vivo* study, undiluted technical 2-phenoxyethanol is an irritant to the rabbit eye.

Skin sensitisation:
In a Guinea Pig Maximisation Test, 2-phenoxyethanol was not a skin sensitiser.
Data from several studies in patients with suspected allergic contact dermatitis indicated that the prevalence of skin sensitisation by 2-phenoxyethanol in cosmetic products is low.

Dermal absorption
In a properly-conducted guideline study with human skin samples, dermal absorption of 2-phenoxyethanol with concentrations of 0.2% and 1.0% was determined in a rinse-off and a leave-on formulation. Around 36% dermal absorption was determined for the rinse-off formulation, whereas around 80% was found for the leave-on formulation. In both formulations, the dermal absorption was independent of the test substance concentration used. Mean values + 1 SD are: 37 + 10, i.e. **47%** dermal absorption for 1% 2 phenoxyethanol in rinse-off formulations and 78 + 7%, i.e., **85%** for 1% 2 phenoxyethanol in leave-on formulations. Use of this data for the calculation of a systemic exposure dose (SED) is not necessary since calculation of the Margin of Safety is based on a dermal toxicity study. Dermal absorption in rats in vivo was determined as 75-76% when 2-phenoxyethanol was applied in a concentration of 1% (37 µg/cm^2) in two sunscreen lotions (AR 9).

Repeated dose toxicity
The following table gives an overview on the RDT studies with 2-phenoxyethanol.

Table 13 Summary of repeated dose toxicity studies on 2-Phenoxyethanol

Study	Species and exposure conditions (oral and dermal doses: mg/kg bw/day)	Critical target organs or effects	Dose descriptor (mg/kg bw/day)	Remarks
Inhalation:				
BASF AG, 2007, Ref. 3	Wistar rat, nose only, 14 days; 0, 48.2, 246, and 1070 mg/m^3. 6 h/day, 5 days per week	Airways: local effects, irritation, increase of lung weight at mid dose	NOAEC: 48.2 x 5/7 = 34.4 mg/m^3	GLP study, OECD 412
Dermal studies:				
Dow Chemicals, 1984, (ref 17)	New Zealand White rabbit, dermal, occlusive (24 h/day); days 6 - 18 of gestation; 0, 300, 600 and 1000 mg/kg bw/day	Only local effects on skin and weight loss in females	NOAEL: 600 (NOAEL offspring: 1000)	Dose range finding study with 10 dams per dose group
Scortichini et al., 1987, (ref 18)	New Zealand White rabbit, dermal, occlusive (24 h/day); days 6 - 18 of gestation; 0, 300, 600 and 1000 mg/kg bw/day	Haematotoxicity, 5 deaths of dams at 600 and 9 deaths at 1000 mg/kg bw/day	NOAEL: 300 (NOAEL offspring: 600)	Published report
Dow Chemicals, 1986, (ref. 13); Breslin et al. 1991, (ref. 12)	Rabbit, dermal, 90 days. 0, 50, 150, 500 mg/kg bw/day; 5 days per week	Only local effects on skin	NOAEL: 500 x 5/7 = 357	GLP study, equivalent to OECD guideline 411. Also published report.

Oral studies:				
Breslin et al. 1991, Ref. 12	Rabbit, oral, gavage, 10 days, 0, 100, 300, 600, 1000 mg/kg bw/day	Haematotoxicity in all dose groups	LOAEL: 100	Published report
BASF AG, 2006, (ref. 16)	Wistar rat, oral, gavage, days 6 - 19 of gestation. 0, 100, 300 and 1000 mg/kg bw/day	T-Bilirubin, total proteins ↓; Triglycerides ↑; (no haemato-toxicity)	NOAEL: 300 (NOAEL offspring: 1000)	GLP study, OECD 414. Additionally: clinical chemistry and haematotoxicity parameters
Bayer AG, 2002 (ref. 10)	Wistar rat, oral, 90 days, diet. Doses of males (M): 0, 34, 169, 697. Doses of females (F): 0, 50, 234, 939.		NOAEL: 697 (males)	GLP study but considerable decrease of body weight, food and water intake in all groups including the control group. → Study not reliable
Unilever, 1993 (ref. 11)	Wistar rat, oral, 90 days, diet. Doses M+F combined: 0, 40, 81, 164, 419	Males: Liver: organ weight and functional parameters reduced at 164 mg/kg bw/day	NOAEL: 164	Summary report and data tables available. GLP study downgraded to non-GLP for unclear reasons. →Study not reliable
NIPA Labs, 1977 (ref. 40)	SD rat, oral, 90 days, gavage. Doses M+F: 0, 80, 400, 2000.	Males: inflammation of kidney at mid dose	NOEL: 80	Pre-GLP study, several non-treatment related deaths in various dose groups, purity of test substance unknown. → Study not reliable
MHLW Japan, 2002 (subm. II, ref.1)	F344 rat, oral, 90 days, drinking water. Doses M: 0, 96, 185, 369, 687, 1514; Doses F: 0, 163, 313, 652, 1000, 1702	Haematotoxicity, kidney, bladder	NOAEL: 369 (males)	GLP study. Complete study report not available, only summary report and summary appendices (complete data set)
MHLW Japan, 2002 (subm. II, ref.2)	B6D2F1 mouse, oral, 90 days, drinking water. Doses M: 0, 182, 390, 765, 1178, 2135 Doses F: 0, 236, 478, 948, 1514, and 2483	Liver	NOAEL: 390 (males)	GLP study. Complete study report not available, only summary report and summary appendices (complete data set)
MHLW Japan, 2007 (subm. II, ref.3)	F344 rat, oral, 2 years, drinking water. Doses M: 0, 124, 249, and 510 Doses F: 0, 191, 380, and 795	Histological kidney effects (males) Dose-dependent increases in ovary weights in all dose groups	NOAEL 249 (males)	GLP study. Complete study report not available, only summary report and summary appendices (complete data set)
MHLW Japan, 2007 (subm. II, ref.4)	B6D2F1 mouse, oral, 2 years, drinking water. Doses M: 0, 468, 898, and 1701 Doses F: 0, 586, 1072, 2058	Serum cholesterol ↓, phospholipids, and triglycerides in males ↓; body weight gain in both sexes ↓	NOAEL: 468 (males) 586 (females)	GLP study. Complete study report not available, only summary report and summary appendices (complete data set)
NTP 1984 (ref. 15); Heindel et al., 1990 (ref. 14)	Fertility study: CD-1 mouse, oral, F0, 126 days, diet Doses M: 0, 400,	Liver weight increase	NOAEL: 400 (males) 950 (females)	GLP study

	2000, 4000. Doses F: About twice male doses			

Inhalation

A 14-day inhalation study with rats was conducted nose/head-only, according to the OECD guideline, with nominal concentrations of 0, 40, 200 and 1000 mg/m³. The animals were exposed 6 hrs/day and 5 days/week. Besides decreased body weight gain (females only) and food consumption (males and females) at the highest concentration, treatment-related effects were restricted to irritation in nasal cavity, larynx and lung and increased lung weights in mid- and high-dose animals. Taking intermittent exposure with a factor of 5/7 into account, a NOAEC of 34.4 mg/m^3 may be used.

Inhalation of the evaporated substance after application of cosmetic products is possible. In a worse-case shower scenario (room volume 10 m^3, 50% evaporation, exposure time 30 min, 50% inhalation of evaporated substance), using 13.3 g shower gel containing 1% 2-phenoxyethanol (19 g/day, frequency 1.43), 1.4 mg/day of the substance may be bioavailable by inhalation. This amount is considered low and covered by the MoS.

Dermal exposure

Three repeated-dose toxicity (RDT) studies in rabbits with dermal exposure are available. According to WHO (AR 5), dermal absorption by rabbit skin is high (similar to rat) compared with human skin, which means a conservative approach when such studies are used for human safety assessment. Dermal RDT studies in rats or mice are not available.

In a pilot study for developmental toxicity in rabbits (days 6 – 18 of gestation; 0, 300, 600 and 1000 mg/kg bw/day), no systemic effects occurred up to 1000 mg/kg bw/day (ref. 17). In the developmental toxicity study at the same dosing schedule, however, deaths obviously due to haematoxicity were observed: 9 dead or moribund animals at 1000 mg/kg bw/day, 5 at 600 mg/kg bw/day, and no deaths at 300 mg/kg bw/day. Most deaths occurred between gestation days 11 and 18 (6 to 13 doses). No signs of maternal toxicity were seen at the low dose so that the maternal NOAEL of this study is 300 mg/kg bw/day (the NOAEL for developmental toxicity was concluded to be 600 mg/kg bw/day) (ref. 18).

The study report of the original main study (AR 6) was not provided; only a published report is available (ref. 18). Breslin et al. (1991, ref. 12), the authors of the 90-day dermal toxicity study in rabbits (see below), reported that according to their experience from additional pilot studies conducted with non-pregnant rabbits at dermal doses of 1000 mg/kg bw/day for 14 days, the susceptibility of individual rabbits to the haemolytic effects at high doses of dermally applied 2-phenoxyethanol was variable, with many animals exhibiting no haematolytic effects. However, once clinical signs developed, death followed rapidly, usually within 24 hrs (subm II, ref. 1; AR 7, quoted by subm II, ref. 1 and by ref. 18).

In a 90-day dermal study with rabbits with doses of 0, 50, 150 and 500 mg/kg bw/day under occlusion (5 days a week), apart from sporadic and slight skin irritation no other effects were observed, in particular no haematotoxicity (ref. 12, 13). The SCCS considers this study as a key study for the safety assessment of 2-phenoxyethanol because the dermal route is the relevant route for 2-phenoxyethanol as a cosmetic ingredient and the rabbit has been shown to be the most sensitive species to haematotoxic effects when orally exposed to 2-phenoxyethanol. 2-Phenoxyethanol is the toxic agent for haematotoxicity whereas the main metabolite 2-phenoxyacetic acid did not show haematotoxic effects *in vitro* (ref. 12, 36, 37).

The French Agence Nationale des Sécurité du Médicament et des Produits de Santé (ANSM; AR 15) has stated that this study does not comply with the requirements of OECD Testing Guideline no. 411, since the animals were in contact with the test substance for 5 days a week rather than 7 days a week. However, the OECD Testing Guideline states the following: "The animals are treated with the test substance, ideally for at least 6 hours per day on a 7-

day per week basis, for a period of 90 days. However, based primarily on practical considerations, application on a 5-day per week basis is considered to be acceptable." The 90-day dermal toxicity study conducted with 2-phenoxyethanol is therefore considered acceptable within the OECD guidelines. To account for the dosing schedule used in this study, the NOAEL of 500 mg/kg bw/day should be multiplied by a factor of 5/7 to give an adjusted NOAEL of 357 mg/kg bw/day.

Oral exposure
Several oral studies from three species are available: rat, mouse and rabbit, rabbit being the most sensitive species of these three species.

Rabbits received 2-phenoxyethanol orally by gavage at 100, 300, 600, or 1000 mg/kg bw/day for 10 days. While 1 animal died in the 300 mg/kg dose group, none of the animals in either high dose group died due to severe haematotoxicity. Signs of haematotoxicity were observed in all dose groups. In rabbits gavaged with 100 or 300 mg/kg bw, treatment-related microscopic changes were observed in the spleen. The LOAEL of this study is 100 mg/kg bw/day.

A 90-day guideline study in rats from 2002 was conducted with 0, 500, 2500 and 10,000 ppm in diet. The NOAEL was concluded to be the high dose of 10,000 ppm in diet corresponding to an intake of 697 mg/kg bw/day in males and 939 mg/kg bw/day in females (ref. 10). However, in control groups and in all treatment groups, parameters of food intake, water intake and body weight gain declined during the study and persisted during the recovery period. These unusual data were not commented upon and not explained. It is assumed that the animals of this study were ill, potentially due to an infection. The study is considered of limited value and not reliable.

On the basis of a pilot study in rats with dosing of 0%, 0.05%, 0.10%, 0.20% and 0.50% of the test substance in the diet resulting in no severe effects, a 90-day guideline study with the same dosing scheme corresponding to 0, 40, 81, 164 and 419 mg/kg bw/day (mean combined male and female intake) was conducted. However, an amendment to the study plan indicated that only a non-GLP summary report would be produced because the study was "deemed to be a low priority study for which full reporting will not be necessary" (ref. 11). The study documentation is incomplete. Methodology used is not described. Analytical data on the purity of the test substance that might be critical regarding study evaluation is not available.
In conclusion, the study is considered of limited value because of incomplete study documentation and unclear circumstances such as the unusual discontinuing of a GLP study and downgrading to a non-GLP status and the late study summary report, i.e. 5 years after the in-life phase.

A 90-day oral study in rats dated from 1977 (pre-GLP) was reported with dosing by gavage of 0, 80, 400, 2000, mg/kg bw/day. Deaths of five animals during treatment, for unknown reasons, which were probably not treatment-related, suggest poor husbandry or animal treatment in the test facility. The purity of the 2-phenoxyethanol batch used in this study is unknown. The study is considered of limited value.

In the **second submission** of data in 2015, four summary reports of drinking water studies in rats and mice conducted in Japan (2002-2004) were provided together with a complete set of study tables, two 90-day studies and two carcinogenicity studies (subm II, ref. 1, 2, 3 and 4).
In the 90-day study in rats, the dose levels were 0, 1250, 2500, 5000, 10,000 and 20,000 ppm in drinking water; in the corresponding carcinogenicity study in rats, dose levels of 0, 2500, 5000, and 10,000 ppm were used.
In the 90-day study, the critical effects in this study are the effects on red blood cell parameters and the histopathological changes in the kidney and urinary bladder which occurred at doses ≥10,000 ppm. The NOAEL is considered to be 5000 ppm corresponding to

369 mg/kg/day in males and 652 mg/kg/day in females. The LOAEL is 687 mg/kg/day in males and 1000 mg/kg/day in females.

In the carcinogenicity study, there was no evidence of carcinogenic activity of 2-phenoxyethanol in male or female rats and there were no clear treatment-related haematological effects. The kidney was a target organ in males in this study with an increased incidence of slight to moderate urothelial hyperplasia and slight papillary mineralisation and necrosis observed in males at 10,000 ppm. No histopathological findings in the kidney were observed in females. Based on the histopathological findings in the kidney in males, the NOAEL is established as 5000 ppm corresponding to 249 mg/kg/day.

In the 90-day study in mice, the dose levels were 0, 1250, 2500, 5000, 10,000 and 20,000 ppm in drinking water, in the corresponding carcinogenicity study in mice, dose levels of 0, 5000, 10,000 and 20,000 ppm were used.
Changes in red blood cell parameters in females (haemoglobin, MCHC, and MCV) and males (reticulocytes) at 20,000 ppm suggest a slight haemolytic anaemia at the high dose but no effect at lower doses. Changes in clinical chemistry parameters may suggest a treatment-related effect on the liver including decreases in cholesterol and phospholipid at doses of > 5,000 ppm in males, although there was no evidence of any histopathology in the liver and no increase in liver enzymes (GPT, GOT). Relative increases of kidney weight occurred in both sexes at higher dose levels (≥10,000 ppm). While it is possible that the liver-related changes in cholesterol and phospholipid are not adverse, these findings in males are conservatively used as the basis for establishing the NOAEL in this study at 2500 ppm, corresponding to an intake of 390 mg/kg/day.
In the carcinogenicity study, also conducted in drinking water, there was no evidence of carcinogenic activity of 2-phenoxyethanol in male or female mice. There was no evidence of any histopathology in the liver and no increase in liver enzymes (ALT, AST). However, similar effects on cholesterol and phospholipids were observed in the 13-week drinking water study in the same strain of mice. Therefore, these findings in males in the chronic study were conservatively concluded to be treatment related. Furthermore, the significant effects on body weight gain indicate that the maximum tolerated dose was exceeded in males at 10,000 ppm in males (body weight 84% of controls) and in females at 20,000 ppm (body weight 79% of controls). Therefore, the NOAEL for 2-phenoxyethanol in this study was concluded to be 5000 ppm in drinking water, corresponding to an intake of 468 mg/kg bw/day in males and 586 mg/kg bw/day in females.

Conclusion on RDT studies
The available oral studies indicate that the rabbit is the most sensitive of the species tested, mainly due to its susceptibility to haematotoxic effects. Target organs in rats and mice were the kidney and liver, probably due to an extensive first-pass metabolism and formation of high amounts/concentrations of 2-phenoxyacetic acid in the systemic circulation (see section 3.3.9). Haematotoxicity in rats and mice was less pronounced than in rabbits and was not observed or doubtful in the long-term studies, probably due to adaptation over time. Data suggest that mice are somewhat more resistant to the toxic effects of 2-phenoxyethanol and its main metabolite 2-phenoxyacetic acid than rats. However, no dermal RDT studies were conducted with rats and mice although dermal exposure is the usual exposure route for most cosmetic ingredients such as 2-phenoxyethanol and more relevant than oral exposure, which is often used as a surrogate exposure when animal safety studies are conducted with cosmetic ingredients. This view on the limited relevance of oral RDT data and dermal exposure as the relevant exposure route is supported by the toxicokinetic data (section 3.3.9), where an extensive first-pass metabolism after oral exposure of rats was found so that only traces of the haematotoxic agent 2-phenoxyethanol reached the systemic circulation. In contrast, after dermal exposure of rats even to low doses of 2-phenoxyethanol, up to 20% of the dose appeared in the blood as the parent compound (Kim et al. 2015, AR 9). Therefore, the 90-day dermal toxicity study in rabbits

with an adjusted **NOAEL of 357 mg/kg bw/day** is used as the key study for the MoS calculation.

Mutagenicity

2-Phenoxyethanol was tested for mutagenic/genotoxic potential in an adequate battery of *in vitro* and *in vivo* tests with various endpoints. 2-Phenoxyethanol was not mutagenic in the Ames test at concentrations up to 5000 µg/plate with and without metabolic activation. Further tests for gene mutation at the *Hprt* locus in mammalian cells also showed no evidence of mutagenic potential either with or without metabolic activation. *In vitro* chromosome aberration tests showed no evidence of a clastogenic effect.

In vivo tests also showed no mutagenic/genotoxic potential for 2-phenoxyethanol. *In vivo* micronucleus and chromosome aberration tests conducted in mice and rats showed no evidence of clastogenic potential. Testing for DNA damage in an UDS test in rats also showed no evidence of genotoxicity.

Consequently, based on the present reports, 2-phenoxyethanol can be considered to have no *in vivo* genotoxic potential and to be without genotoxic hazard to humans. Additional tests are unnecessary.

Carcinogenicity

Two summary reports from studies in rats and mice are available (for exposure route and doses, see the discussion on RDT studies). No neoplastic lesions were detected (subm II, ref. 3 and ref. 4).

Reproductive toxicity

A two-generation reproductive toxicity study in mice with 0, 0.25, 1.25 and 2.5% 2-phenoxyethanol in the diet was conducted according to an NTP protocol. Fertility was only minimally affected at the highest dose, but evidence of significant toxicity to the offspring was observed when 2-phenoxyethanol was administered at the mid- and high-dose level. Parental toxicity was reported at the mid- and high-dose level. For both males and females, the NOAEL for parental toxicity and reproductive toxicity was concluded to be the low dose, i.e. 0.25% in diet. For males, a NOAEL of 400 mg/kg bw/day was calculated. For females, the NOAEL was approximately 950 mg/kg bw/day.

Two studies on developmental toxicity are available, one oral rat and one dermal rabbit study. In both studies, the NOAELs for developmental toxicity were higher than the NOAELs for maternal toxicity (300 mg/kg bw/day in both studies).

Toxicokinetics

2-Phenoxyethanol is rapidly absorbed, distributed, metabolised and excreted. More than 90% of the dose recovered was excreted in urine, mainly as 2-phenoxyacetic acid, independent of the exposure route (rats, i.v., dermal, oral; humans, dermal or oral). This metabolite is formed in a two-step oxidation process by cytosolic alcohol dehydrogenase (ADH; EC 1.1.1.1.) and aldehyde dehydrogenase (ALDH; 1.2.1.3), in rats mainly in the liver but also in the skin (5-7% of the rate of liver; ref. 9).

In a study on species differences *in vitro*, the rate of metabolism of 2-phenoxyethanol to 2-phenoxyacetic acid was highest in liver S9 homogenates from human donors followed by rat > mouse > rabbit. As 2-phenoxyethanol (and not 2-phenoxyacetic acid) is the agent responsible for haematoxicity observed *in vivo* (ref. 12; ref. 36), limited capacity of metabolising 2-phenoxyethanol by rabbits may at least in part explain the prominent susceptibility to haematotoxic effects after oral 2-phenoxyethanol exposure compared to rats (see RDT studies and ref. 37).

After oral exposure of rats by gavage of radiolabelled 2-phenoxyethanol up to 1000 mg/kg bw, most of ^{14}C was found in urine. The main metabolite 2-phenoxyacetic acid constituted 60-80% of the dose and some of its conjugates (partly ring-hydroxylated) were also found in urine. Only traces of 2-phenoxyethanol could be detected or none at all was found. Radioactivity found in tissues was low, some percentage was excreted in the bile, about 2% was found in faeces and 1-2% exhaled as ethanol (probably an equivalent amount of phenol

was formed), whereas no CO_2 was found in exhaled air (subm II, ref. 6 and 7; ref. 27, ref. 28).
Plasma kinetics of rats after single oral dosing by gavage in a range between 30 and 1000 mg/kg bw showed linear increases of C_{max}, similar T_{max} and similar half-life values. Only in females, half-life values were slightly enhanced at doses ≥300 mg/kg bw. The bioavailability of ^{14}C-2-phenoxyethanol was comparable at the dose of 40 and 400 mg/kg bw. Plasma kinetics demonstrated a disproportional increase of the AUC values with increasing dose, indicating a saturation of excretion.

In a recently published study, after dermal or i.v. exposure of rats, 2-phenoxyethanol and its major metabolite, 2-phenoxyacetic acid, were simultaneously analysed in rat plasma, urine and several tissues. After application of a low topical dose of 2.34 mg/kg PE (corresponding to around 37 µg/cm^2) in sunscreen formulations, the proportion of free 2-phenoxyethanol reached around 20% of the bioavailable dose in serum (Kim et al. (2015), ref. AR 9), much higher than after oral exposure of rats at much higher doses (ref. 27 and ref. 28).

After oral exposure of rabbits by gavage of 2-phenoxyethanol, up to 16% of the bioavailable dose in serum consisted of free and conjugated 2-phenoxyethanol (ref. 12), i.e. much more than in rats (concentrations below the LoQ in serum and 0.07% in urine; ref. 27 and ref. 28). Although most RDT studies in rabbits were performed by dermal exposure, no toxicokinetic data via this route is available.

In humans, at low doses of 2-phenoxyethanol after oral exposure of an adult male or in clinical trials with adults or pre-term babies after dermal exposure, only 2-phenoxyacetic acid and its conjugates was detected in urine (detection limit of 2-phenoxyethanol 0.5 - 2 ppm). In biomonitoring studies of consumers, only 2-phenoxyacetic acid was detected in 24 h urine up to 28 mg/L (95-percentile). Higher amounts found in women compared with men suggest that the main exposure is by 2-phenoxyethanol in cosmetic products. From these studies, no firm conclusions can be drawn on the systemic availability of 2-phenoxyethanol in humans after dermal exposure.

The PBPK model provided by Troutman et al. (2015, subm II, ref. 12) was mainly developed by use of oral rat kinetic data including available human clinical and biomonitoring data. The model is primarily focused on the formation of 2-phenoxyacetic acid and kidney toxicity, which is probably caused by rapid formation of this metabolite in the liver as a first pass effect. During the commenting phase of the Opinion, modified data was provided by the applicant using the additional toxicokinetic data after topical exposure of rats (Kim et al. (2015, ref. AR 9). The updated PBPK data for the dose metric AUC of phenoxyethanol in adult humans (Table 11) were around 3-fold higher than those predicted by Troutman et al. (2015, subm II, ref. 12; Table 8) resulting in MoS values of around 30 for adults when comparing AUC_{Phe} in human adults with the respective AUC value in rats at the NOAEL (Table 8). No supplementing data were provided for infants/children exposure groups, for which the resulting MoS values may be around 10-15.

The rat PBPK model can reproduce the chemical-specific pharmacokinetic data under various experimental conditions for oral route and dermal application and is reliable with regard to its predictions of dose metrics (C_{max} AUC, renal clearance).

Regarding the human PBPK model, the PBPK model can reproduce the chemical-specific pharmacokinetic data under two experimental conditions for oral route and dermal application and is reliable with regard to its predictions of only one dose metrics: cumulative PhAA in urine. The human data (cumulative urine excretion of PhAA) were used for calibration of the human PBPK model. Unfortunately, comparison between AUC (PhE or PhAA) observed and AUC (PhE or PhAA) predicted was not performed, which limits the applicability of the PBPK modeling for humans.

The SCCS considers that the human PBPK model cannot be used for the current evaluation for two reasons:
- The dose metric considered for the Margin of Safety (MOS) calculations is AUC, whereas no measured data in human blood is available. Consequently, no validation for the human PBPK model concerning the selected dose metric (AUC) is available. The confidence in the human PBPK model is considered low for this relevant (AUC) internal dose metric.
- The human data (cumulative urine excretion of PhAA) were used for calibration of the human PBPK data. The lack of human data for validation (comparison between observed and predicted for cumulative excretion) limits the applicability of the PBPK modeling for humans.
In the biomonitoring studies used by Troutman et al. (2015, ref. AR 12), the dose levels were unspecified, i.e. external exposure was estimated but not measured. SCCS considers that the human PBPK model (once validated) could be used to estimate a corresponding external exposure from measured PhAA concentrations in urine, but not to verify the model.

Under these considerations, additional model evaluation would be needed regarding the reliability (model testing, uncertainty and sensitivity) as described and recommended by WHO (2010, figure 10; ref. AR 8) as well as additional information on the relationship between blood concentration, AUC and renal clearance.

Human data
Contact sensitisation in humans has been documented, but from the available studies it can be concluded that this is rare. The risk of becoming sensitised is very low.
Human biomonitoring data are discussed in the Toxicokinetics section. Since 2-phenoxyacetic acid is the main metabolite of 2-phenoxyethanol in humans, data on background levels of 2-phenoxyacetic acid in human urine samples suggest that cosmetic products are a major source of 2-phenoxyethanol exposure to consumers.

Aggregate exposure to 2-phenoxyethanol from cosmetic sources
Cosmetics, which contain 2-phenoxyethanol as a preservative up to a concentration of 1% and which expose users to this substance via the dermal route are considered to account for a dose of 2.69 mg/kg bw/day, whereas the inhalation route (shower scenario) may contribute an additional 1.4 mg/day corresponding to 0.02 mg/kg bw/day. 2-Phenoxyethanol as used as a fragrance in fragrance mixtures in cosmetics may account for around 0.05 mg/kg bw/day.
Taken together, the aggregate exposure to 2-phenoxyethanol in cosmetics may sum up to **2.76 mg/kg bw/day** resulting in a **MoS of 130**.
The oral intake of 2-phenoxyethanol via exotic fruits such as avocados is unknown but is considered negligible due to the rapid first-pass effect in the human liver.

2-Phenoxyethanol exposure of children
For children of ≤3 years of age, the French authority ANSM [1] (2012, ref. AR 15) calculated the specific exposure to cosmetic products with an assumed concentration of 1% 2-phenoxyethanol in the products. Reasonable worst case data of exposure and an unrealistic default body weight of 3.4 kg (for neonates) were used. From all toddler/infant-specific product types, ANSM calculated an SED value of 16.7 mg/kg bw/day and a MoS of 10 by use of an external NOAEL of 164 mg/kg bw/day (derived from ref. 11).
From their data, a reasonable worst case value of 61.2 mg 2-phenoxyethanol per day can be calculated as an external aggregate exposure to age-specific cosmetic products. When using a low default body weight of 9 kg for toddlers/infants ≤3 years (5-percentile; EFSA 2012, AR 16), the daily external exposure is 6.8 mg/kg bw/day and the **MoS** is **53** when using the NOAEL of 357 mg/kg bw/day from the 90-day dermal toxicity study in rabbits.

[1] Agence nationale de sécurité du médicament et des produits de santé

When using an even lower body weight default value of EFSA (7 kg, 5-percentile) and the NOAEL of 357 mg/kg bw/day, a daily dose of 8.7 mg/kg bw/day and a **MoS** of **41** results.

In conclusion, children ≤3 years may be higher exposed to 2-phenoxyethanol in cosmetic products than adults. However, the MoS values derived from reasonable worst case scenarios are considered sufficient for this age group.

Overall safety assessment of 2-phenoxyethanol

Haematotoxicity is a predominant toxicological feature of 2-phenoxyethanol *in vivo* and *in vitro*. Comparison of oral studies in rats, mice and rabbits indicates that rabbits are the test species most sensitive to haematotoxic effects by 2-phenoxyethanol. The main metabolite 2-phenoxyacetic acid is formed in rabbit liver at much lower rates than in humans > rats > mice. Rabbit erythrocytes were found less resistant to 2-phenoxyethanol toxicity than erythrocytes from humans > rats > mice, whereas 2-phenoxyacetic acid did not show a haematotoxic potential. Systemic availability of 2-phenoxyethanol after oral exposure of rats is very low due to a strong first pass effect in rat liver and the rapid formation of the main metabolite 2-phenoxyacetic acid, which may accumulate in the kidney and may be responsible for kidney toxicity in rats after oral exposure. In contrast, dermal exposure of rats to 2-phenoxyethanol revealed much higher concentrations of the parent compound in blood than after oral exposure. This may also be true for other species such as humans. For these reasons and because dermal exposure is the relevant route of exposure of humans to 2-phenoxyethanol in cosmetic products, preference is given to dermal studies in rabbits, the most sensitive species tested, whereas the oral route is considered of questionable relevance.

Given the much higher capacity of humans to metabolise 2-phenoxyethanol compared with rabbits, the toxicokinetic default factor of 4.0 can be reduced to 1.0 yielding a minimum Margin of Safety (**MoS**) of **25** instead of 100 for the safety assessment of 2-phenoxyethanol. Using an adjusted NOAEL of 357 mg/kg bw/day from a 90-day dermal RDT study in rabbits and the aggregate exposure for preservatives in cosmetics, for adult humans a **MoS of 130** was derived. The large span of this MoS to the MoS of 25 selected by the SCCS for the safety assessment also covers the safety of infants and babies to 2-phenoxyethanol exposure in cosmetic products.

4 CONCLUSION

1. *Does SCCS consider Phenoxyethanol safe for use as a preservative with a maximum concentration of 1.0 %, taking into account the information provided?*

   The SCCS considers 2-phenoxyethanol safe for use as a preservative with a maximum concentration of 1.0%, taking into account the information provided.

2. *The SCCS is asked, when making the assessment, to take into account the specific age groups who might be particularly susceptible to the effects of Phenoxyethanol used as preservatives in cosmetic products.*

   The toxicokinetics default factor of 4.0 can be reduced to 1.0 yielding a minimum Margin of Safety (MoS) of 25 instead of 100 for the safety assessment of 2-phenoxyethanol.
   Therefore, the MoS of about 50 for children also covers this specific age group who might be higher exposed to 2-phenoxyethanol than adults.

3. *Does the SCCS have any further scientific concerns with regard to the use of Phenoxyethanol in cosmetic products?*

   This Opinion does not take into account exposure from sources other than cosmetics.

5 MINORITY OPINION

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6 REFERENCES SUBMISSION I

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9 Annex 1

PBPK Modelling of of 2-phenoxyethanol and its metabolite 2-phenoxyacetic acid in rats and humans

Troutman et al. (2015, Subm II, ref. 10)

■ Table 1: Chemical-Specific Input Parameters by Species

Physico-Chemical inputs	***Abbrev.***	***Units***	***Values***		
Phenoxyethanol_MW	MW_PE	g/mol	138.17		
Phenoxyethanol_Log Kow	Log_Kow_PE	unitless	1.16		
Phenoxyethanol_fraction unbound	fup_PE	unitless	0.56		
Phenoxyacetic acid_MW	MW_PAA	g/mol	152.15		
Phenoxyacetic acid_Log Kow	Log_Kow_PAA	unitless	1.34		
Phenoxyacetic acid_fraction unbound	fup_PAA	unitless	0.21		
Partition Coefficients for Phenoxyethanol			**Rat**	**Rabbit**	**Human**
Blood:Air	Pb_PE	unitless	300815	300815	300815
Fat:Blood	Pf_PE	unitless	0.660	0.660	0.660
GI:Blood	Pgi_PE	unitless	0.999	0.999	0.999
Kidney:Blood	Pk_PE	unitless	0.793	0.793	0.793
Liver:Blood	Pl_PE	unitless	0.771	0.771	0.771
Lung:Blood	Plu_PE	unitless	0.871	0.871	0.871
Skin:Blood	Psk_PE	unitless	1.122	1.122	1.122
Richly Perfused:Blood	Pr_PE	unitless	0.897	0.897	0.897
Slowly Perfused:Blood	Ps_PE	unitless	0.100	0.100	0.100
Partition Coefficients for Phenoxyacetic Acid					
Blood:Air	Pb_PAA	unitless	369685	369685	369685
Fat:Blood	Pf_PAA	unitless	0.078	0.078	0.078
GI:Blood	Pgi_PAA	unitless	0.267	0.267	0.267
Kidney:Blood	Pk_PAA	unitless	3.5	3.5	3.5
Liver:Blood	Pl_PAA	unitless	0.680	0.680	0.680
Lung:Blood	Plu_PAA	unitless	0.325	0.325	0.325
Skin:Blood	Psk_PAA	unitless	0.348	0.348	0.348
Richly Perfused:Blood	Pr_PAA	unitless	0.175	0.175	0.175
Slowly Perfused:Blood	Ps_PAA	unitless	0.582	0.582	0.582
Oral Absorption/Dissolution Rates					
Absorption Rate of PE	ka_PE	hr-1	3.5	3.5	3.5
Dissolution Rate of PE (in GI)	Ka_PE_dissolution	hr-1	1.0 (0.3)[a]	1.0	1.0
Solubility of PE (in GI)	PE_Solubility	mg/L	27000 (2700)[b]	27000	27000
Dermal Specific descriptors					
Dermal Penetration of PE	Kp_PE	cm/hr	0.0025	0.0025	0.0025
Surface Area	SA	cm^2	Simulation-specific		
Volume Applied to skin	Appl_Vol	cm^3	Simulation-specific		
Competitive Loss of Chemical from skin	K_Loss_PE	cm/hr	0.0 (0.005) [c]	0.0 (0.005) [c]	0.0 (0.005) [c]
Metabolic Rates					
Vmax for Phenoxyethanol (Liver)	Vmax_Metab_PE	mg/hr/(kg bw^0.75)	562	51	327
Km for Phenoxyethanol (Liver)	Km_Metab_PE	mg/L	127	1.0	40
Vmax for Phenoxyethanol (GI)	Vmax_GIMetab_PE	mg/hr/(kg bw^0.75)	-	500	-
Km for Phenoxyethanol (GI)	Km_GIMetab_PE	mg/L	-	1.0	-
Vmax for Phenoxyacetic acid (Liver)	Vmax_Metab_PAA	mg/hr/(kg bw^0.75)	75	0.0	7.5
Km for Phenoxyacetic acid (Liver)	Km_Metab_PAA	mg/L	500	500	500
Active Secretion of PAA by Kidney					
Vmax for Secretion of Phenoxyacetic acid	Vmax_Secretion	mg/hr/(kg bw^0.75)	30	-	30
Km for Secretion of Phenoxyacetic acid	Km_Secretion	mg/L	100	-	100

[a] Dissolution rate of PE in GI tract of orally-dosed rats was assigned a value of 1.0 hr^{-1} in all simulations except BASF, 2007 in which 0.3 $hr^{-1}$was found optimal.

[b] Solubility of PE in GI tract of orally-dosed rats was assigned a value of 27000 mg/L in all simulation except BASF, 2007 in which 2700 mg/L was found optimal.

[c] Competitive Loss of Chemical from Skin Application Site (e.g. volatilization, physical removal) is assigned a value of either 0.0 cm/hr (occluded application site) or 0.005 cm/hr (non-occluded application site)

Table 2: Physiological Input Parameters used in the PBPK Model for PhE and PhAA in Rats, Rabbits and Humans

Parameter	***Abbrev.***	***Units***	**Rat**	**Rabbit**	**Human**
Physiological - Fractional Tissue Volumes					
Body Weight	BW	kg	0.25	2.5	60 (adult) 8 (infant)
Fat	Vfc	frct_BW [a]	0.0675	0.1295	0.2142
GI	Vgic	frct_BW	0.0270	0.0308	0.0171
GI Lumen[d]	Vgiluc	L	0.005	0.005	0.005
Kidney	Vkc	frct_BW	0.0073	0.0047	0.0044
Liver	Vlc	frct_BW	0.0366	0.0280	0.0257
Lung	Vluc	frct_BW	0.0050	0.0065	0.0076
Muscle	Vmc	frct_BW	0.4030	0.4109	0.4000
Skin	Vskc	frct_BW	0.1903	0.1209	0.0371
Richly Perfused	Vrc	frct_BW	0.0143	0.0239	0.0292
Slowly Perfused	Vsc	frct_BW	0.1790	0.1444	0.1217
Fractional Volume of Renal Tubules	Vtuc	frct_Kidney wt	0.1000	0.1000	0.1000
Physiological - Fractional Tissue Flows					
Respiration Rate	Qp	L/hr	7.40	48.0	330
Cardiac Output	Qc	L/hr	4.44	31.8	336
Fat	Qfc	frct_CO [b]	0.0054	0.0604	0.0464
GI	Qgic	frct_CO	0.1014	0.2094	0.1964
Kidney	Qkc	frct_CO	0.1410	0.1350	0.1800
Liver	Qlc	frct_CO	0.1865	0.3340	0.2589
Muscle	Qmc	frct_CO	0.1014	0.1076	0.1339
Skin	Qskc	frct_CO	0.0784	0.0710	0.0536
Richly Perfused	Qrc	frct_CO	0.4574	0.2621	0.2971
Slowly Perfused	Qsc	frct_CO	0.0300	0.0300	0.0300
Urine	Qurc	frct_Kidney [c]	0.1740	0.5400	0.1890
Glomerular Filtration	Gfrc	frct_Kidney	41.04	39.80	24.40 (adult) 14.16 (infant)

[a] Fractional Tissue Volumes expressed as (decimal) Percent of Body Weight

[b] Fractional Blood Flows to Tissues expressed as (decimal) Percent of Cardiac Output

[c] Urinary Production and Glomerular Filtration Rates expressed as (decimal) Percent of Kidney Weight

[d] Vgiluc parameter from literature data of (REF) for rat, and assumed comparable to other species

Table 3: In Vivo and In Vitro kinetic data used to develop and verify the PBPK model

Species	Metric	Sample Matrix	Dose Route	Dose Level (mg/kg)	Purpose	Reference
Rat	PhE, PhAA	Plasma	Oral bolus	152, 456	Calibration	Louisse et al 2010
Rat	^{14}C-PhE	tissue, urine	Oral bolus	40, 400	Calibration	BASF, 2007a
Human	^{14}C-PhE	Urine	Oral bolus	0.152	Calibration	Howes, 1991
Rat, Human skin	^{14}C-PhE	Receptor fluid	In vitro skin penetration		Calibration	Roper et al, 1997
Rat	^{14}C-PhE	Urine	Dermal	18.4, 24.4	Calibration	Howes, 1991
Human	^{14}C-PhE	Urine	Dermal	0.9-7	Calibration	Howes, 1991
Rat	^{14}C-PhE	Urine	Dermal	16, 27, 161	Verification	Howes, 1991
Rat	^{14}C-PhE	Blood	Oral bolus	30, 40, 100, 300, 400	Verification	BASF, 2007a
Human	PhAA	Urine	Dermal	unspecified	Verification	Fromme, 2013 Göen, 2001 Garlantezec, 2012
Human, preterm infants (23-27 GA)	PhE, PhAA	Urine	Dermal	unspecified	Verification	Buhrer, 2002

Table 4: Normalized sensitivity coefficients

Parameter	PhE in Blood	PhAA in Blood	PhAA in Urine
fup_PE	0.0%	0.0%	0.0%
Vmax_Metab_PE	0.3%	0.0%	0.0%
Km_Metab_PE	0.8%	0.1%	0.0%
Pb_PE	0.0%	0.0%	0.0%
Pgi_PE	9.2%	0.1%	0.0%
Pk_PE	0.0%	0.0%	0.0%
Pf_PE	0.7%	0.3%	0.1%
Pl_PE	0.6%	0.0%	0.0%
Plu_PE	0.0%	0.0%	0.0%
Psk_PE	13.8%	2.2%	0.2%
Pr_PE	3.0%	0.2%	0.0%
Ps_PE	**37.7%**	6.7%	0.8%
Ka_PE	**31.6%**	0.9%	0.0%
Ka_PE_dissolution	2.2%	2.0%	0.3%
PE_solubility	0.0%	0.0%	0.0%
fup_PAA	0.0%	14.7%	**81.1%**
Vmax_Metab_PAA	0.0%	0.1%	1.1%
Km_Metab_PAA	0.0%	0.0%	0.1%
Pb_PAA	0.0%	0.0%	0.0%
Pgi_PAA	0.0%	1.9%	0.2%
Pk_PAA	0.0%	0.4%	0.0%
Pf_PAA	0.0%	5.5%	1.0%
Pl_PAA	0.0%	2.3%	0.2%
Plu_PAA	0.0%	0.0%	0.0%
Psk_PAA	0.0%	15.2%	2.7%
Pr_PAA	0.0%	1.0%	0.1%
Ps_PAA	0.0%	**46.0%**	9.5%
Vmax_Secretion	0.0%	0.3%	1.7%
Km_Secretion	0.0%	0.0%	0.3%
GFRc	0.0%	0.1%	0.4%

Table 5: Urinary excretion of PhAA in preterm infants (24-27 GA) following dermal exposure to 2% PhE-containing antiseptic solution.

Predicted concentration in urine (mg/L)	**Observed concentration in urine (mg/L)**
16 (non-occluded) 47 (occluded)	24 (range 5-95)

Table 6: Exposure and application frequency data used in dosimetry predictions in adult humans

Product	**Initial Application Time**	**Exposed Skin Surface Area (cm^2)**	**Application Volume (mL or cm^3)**	**PhE Dose Level (mg/kg/application)**	**No. of Exposures per day**	**Exposure Frequency (hr)**
Shower gel	7:00 AM	17500[a]	0.19	0.0279	1	24
Shampoo	7:00 AM	1440[b]	0.11	0.0151	1	24
Hair conditioner	7:00 AM	1440[b]	0.04	0.0067	1	24
Hair styling	7:30 AM	1010[c]	0.40	0.0574	1	24
Liquid foundation	7:30 AM	565[d]	0.51	0.0790	1	24
makeup remover	7:30 PM	565[d]	0.50	0.0833	1	24
Hand wash soap	8:00 AM	860[e]	0.020	0.00333	10	2.4
Body lotion	7:30 AM	15670[f]	3.910	0.616	2	12
Face cream	7:15 AM	565[d]	0.770	0.121	2	12
Hand cream	8:00 AM	860[e]	1.080	0.164	2	12
Deo non-spray	7:15 AM	200[g]	0.750	0.110	2	12
Eye makeup	7:30 AM	24[h]	0.010	0.00165	2	12
Mascara	7:30 AM	1.6[h]	0.013	0.00210	2	12
Lipstick	7:30 AM	4.8[h]	0.030	0.00450	2	12
Eyeliner	7:30 AM	3.2[h]	0.00250	0.000400	2	12
Toothpaste	8:00 AM	N/A	N/A	0.011	2	12
Mouthwash	8:00 AM	N/A	N/A	0.163	2	12
Total Daily Aggregate exposure (mg/kg/day):				**2.34**	-	-

Product use and exposure information (surface area, application frequency) were calculated using data listed in table 2 or table 4 of the SCCS Notes of Guidance 8th Revision (SCCS/1501/12). All products were assumed to contain a maximum concentration of 1% PhE. [a]total body; [b]hand+1/2 head; [c]1/2 hands+1/2 head; [d]1/2 head (female); [e]hands; [f]total body-head (female); [g]both axillae; [h]not specified

Table 7: Exposure and application frequency data used in dosimetry predictions in 8 kg body weight infants

Product	**Initial Application Time**	**Exposed Skin Surface Area (cm^2)**	**Application Volume (mL or cm^3)**	**PhE Dose Level (mg/kg/application)**	**No. of Exposures per day**	**Exposure Frequency (hr)**
Shampoo	7:00 AM	366[a]	0.0121	0.0151	1	24
Shower gel	7:00 AM	4024[b]	0.0223	0.0279	1	24
Body Lotion	7:30 AM	3292[c]	0.492	0.615	2	12
Face cream	7:30 AM	366[d]	0.193	0.2414	1	24
Face cleanser	12:00 PM	366[d]	0.0666	0.0833	1	24
Body Cleanser	3:00 PM	4024[b]	0.0984	0.123	1	24
Subtotal for Part 1 - Daily Baby Care (mg/kg.day):				**1.46**	-	-
Part 2a (leave-on/nappy)		290[e]	2.11	2.64	1	24
Part 2b (wipes/nappy)		290[e]	0.320	0.400	5	4.8
Subtotal for Parts 2a+2b – nappy (mg/kg/day):				**4.64**	-	-
Total Daily Aggregate exposure for Parts 1+2a+2b (mg/kg/day):				**6.1**	-	-

Product use and exposure information (surface area, application frequency) were obtained from data listed in the SCCS Notes of Guidance 8th Revision (SCCS/1501/12) and US EPA Exposure Factor Handbook (2011) (Ref. 15). All products were assumed to contain a maximum concentration of 1% PhE. [a]1/2 head ; [b] total body; [b]hand+1/2 head; [c]total body-head; [d]1/2 head; [e]urogenital region

Table 8: Exposure comparisons and internal dosimetry predictions

				Internal Dose Metric	
		External dose		AUC (mg*h/L)	
Subpopulation	Description	mg/kg/day	BW	PhE	PhAA
Rat	NOAEL (drinking water)	369	0.25	61.5	690
Adult Human	Aggregate (oral+dermal exposure to cosmetics)	2.34	60	0.924	9.09
Infant/children	Part 1 (Daily Baby Care)	1.46	8	0.171	2.86
Infant/children	Part 2a (Leave-on/nappy)	2.64	8	0.789	13.3
Infant/children	Part 2b (wipes/nappy)	2.00	8	0.598	10.0
Infant/children	Parts 1+2a+2b	6.1	8	1.56	26.2

Table 9: Margin of Safety calculations for aggregate exposures to adult and 8 kg infant/child

	Rat External Oral Dose of PhE at NOAEL (mg/kg/day)		Rat PBPK model→	**Rat Internal dose of PhE at NOAEL** (AUC=mg*hr/L)	
RAT	369			61.5	
HUMAN Subpopulation	**Exposure Scenario**	**Consumer exposure** (mg/kg/day)		**Internal dose from consumer exposure** (AUC=mg*hr/L)	**MoS based on internal dose** (Rat internal dose at NOAEL/ Human internal dose from consumer exposure)
Adult	Aggregate (oral+dermal exposure to cosmetics)	2.34		0.924	67
Infant/Children	Part 1 (Daily Baby Care)	1.46	Human PBPK model→	0.171	359
Infant/Children	Part 2a (Leave-on/nappy)	2.64		0.789	78
Infant/Children	Part 2b (Wipes/nappy)	2.00		0.598	103
Infant/Children	Total (1+2a+2b)	6.1		1.56	39

Figure 1: Diagram of the PBPK model for PhE and PhAA

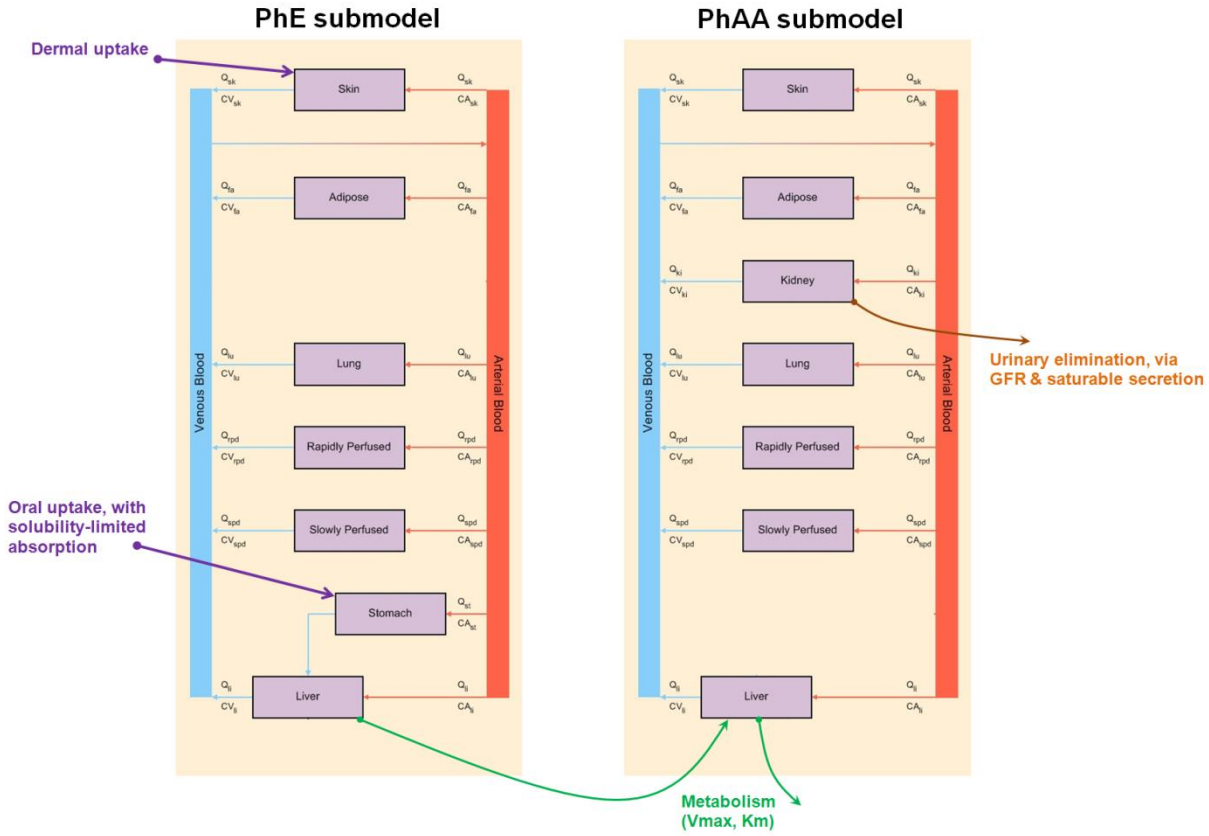

Figure 2: Model-predictions (solid lines) of PhE and PhAA plasma concentrations in rats following a single oral bolus of PhE at 152 mg/kg (Panel A) and 456 mg/kg (Panel B). Experimental (measured) mean concentrations for each analyte from Louisse et al. (2010) are shown as symbols.