Scientific Committee on Consumer Safety

SCCS

SCIENTIFIC ADVICE

on the safety of Thiomersal (CAS No. 54-64-8, EC No. 200-210-4)

and Phenylmercuric salts

as preservatives in cosmetic products

The SCCS adopted this document by written procedure on 2 February 2026

ACKNOWLEDGMENTS

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

For the preliminary version and for the final version of the scientific advice

SCCS members
Dr U. Bernauer
Dr L. Bodin
Prof. Q. Chaudhry (SCCS Chair)
Prof. P.J. Coenraads (SCCS Vice-Chair, Chairperson of the WG)
Dr J. Ezendam
Dr E. Gaffet
Prof. C. L. Galli
Prof. E. Panteri
Prof. V. Rogiers (SCCS Vice-Chair)
Dr Ch. Rousselle
Dr M. Stepnik
Prof. T. Vanhaecke
Dr S. Wijnhoven

SCCS external experts
Dr E. Benfenati
Dr N. Cabaton
Prof. E. Corsini
Dr Ch. Delmaar
Dr A. Koutsodimou
Dr M. Henriqueta D.L.G. Louro
Prof. W. Uter (Rapporteur)
Dr N. von Goetz

This Scientific Advice has been subject to a commenting period of min eight weeks after its initial publication (from 19 November 2025 to 21 January 2026, due to holiday period). No comment was received during this period. The conclusions of this Advice remain but have been slightly reworded.

All Declarations of Working Group members are available on the following webpage:
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1. ABSTRACT

The SCCS concludes the following:

In light of technical and scientific progress and SCCS' expert judgement and taking under consideration in particular the various health concerns, the SCCS is requested:

(1) to identify and justify whether there are specific concerns regarding the current use of 'Thiomersal' and 'Phenylmercuric salts' as preservatives in cosmetic eye products;

Considering that the Margin of Safety based on renal toxicity as the most sensitive endpoint is less than 100, and that genotoxicity evidence is unresolved, the use of 'Thiomersal' and 'Phenylmercuric salts' as preservatives in cosmetic eye products is not considered safe at the concentration levels currently permitted in cosmetic products.

(2) to highlight if there is a potential risk for human health from the current use of 'Thiomersal' and 'Phenylmercuric salts' as preservatives in cosmetic eye products;

The potential risk for human health from use in cosmetic eye products is aggravated by additional exposure to mercuric compounds from sources other than cosmetics.

Keywords: SCCS, scientific advice, preservative, Thiomersal, CAS No. 54-64-8, EC No. 200-210-4, Phenylmercuric salts, Regulation 1223/2009

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SCCS

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

Scientific Committee members

Ulrike Bernauer, Laurent Bodin, Qasim Chaudhry, Pieter Jan Coenraads, Janine Ezendam, Eric Gaffet, Corrado Lodovico Galli, Eirini Panteri, Vera Rogiers, Christophe Rousselle, Maciej Stepnik, Tamara Vanhaecke, Susan Wijnhoven

Contact:
European Commission
Health and Food Safety
Directorate B: Public Health, Cancer and Health security
Unit B3: Health monitoring and cooperation, Health networks
L-2920 Luxembourg
SANTE-SCCS@ec.europa.eu

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Mercury and its compounds have been prohibited from use in cosmetics products already in the Cosmetics Directive 76/768/EEC and this prohibition is still applicable via entry 221 of Annex II to the Cosmetics Regulation (EC) No. 1223/2009.

Nevertheless, entries 16 and 17 of Annex V (preservatives) to the Cosmetics Regulation 1223/2009 allow the use of 'Thiomersal' and 'Phenylmercuric salts (including borate)', respectively, in cosmetic products under specific conditions. In particular, such mercury containing compounds are allowed only in eye products and with a maximum concentration that does not exceed 0.007 % (of mercury), when used alone or in combination (i.e., if mixed with other mercurial compounds that are currently authorised, the maximum concentration of mercury remains fixed at 0.007 %). In addition, cosmetic products that contain such compounds must include in their labelling the warnings 'Contains Thiomersal' or 'Contains Phenylmercuric compounds'.

Apart from 'Thiomersal' that is a single substance, 'Phenylmercuric salts' cover all salts composed of the phenylmercury cation ($C_6H_5Hg^+$) paired with an anion, such as acetate, benzoate, borate, bromide, chloride, oleate, etc.

Mercury and its compounds are toxic, and exposure can lead to various adverse health effects, including but not limited to neurological and kidney damage, skin irritation and allergic reactions, immune and endocrine system disruption, etc. In addition, mercury can accumulate in the body, leading to chronic toxicity even at very low levels of exposure over time. This can affect multiple organ systems, including the digestive, cardiovascular, and reproductive systems.

Given the severe health risks associated with mercury, its safe use in cosmetic products - although already strictly regulated- should be re-evaluated by the SCCS in view of technical and scientific progress. The Commission, therefore, requests the SCCS to provide a scientific advice on the safety of preservatives that contain mercury (and its compounds).

Terms of reference

In light of technical and scientific progress and SCCS' expert judgement and taking under consideration in particular the various health concerns, the SCCS is requested:

(1) to identify and justify whether there are specific concerns regarding the current use of 'Thiomersal' and 'Phenylmercuric salts' as preservatives in cosmetic eye products;

(2) to highlight if there is a potential risk for human health from the current use of 'Thiomersal' and 'Phenylmercuric salts' as preservatives in cosmetic eye products;

3. SCIENTIFIC ADVICE

In the following scientific advice, the abbreviation "THI" will be used for Thiomersal, and "phHg" for Phenylmercuric salts in general, unless the latter are further specified.

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

THI: Thiomersal (INN), (thimerosal, INCI)
phHg: Phenylmercury (use is as salt, see below)

- phHg acetate: Phenyl mercuric acetate (INCI)
- PhHg bromide: Phenyl mercuric bromide (INCI)
- phHg benzoate: Phenyl mercuric benzoate (INCI)
- phHg borate: Phenyl mercuric borate (INCI)
- PhHg chloride: Phenyl mercuric chloride (INCI)

In the following, due to the fact that relevant data are available almost exclusively for thiomersal and phenyl mercuric acetate, only these two mercury compounds (along with methyl mercury; meHg) will be considered. Information on chemical and physical specifications of other phHg compounds is assembled in Annex I "Chemical and Physical Specifications of further phenymercury compounds".

3.1.1.2 Chemical names

THI: Sodium ethylmercury thiosalicylate, Mercurothiolate, Merthiolate, Thiomersalate, o-(Ethylmercurithio)benzoic acid sodium salt, sodium (2-carboxylatophenyl)sulfanyl-ethylmercury, [(o-Carboxyphenyl)thio]ethylmercury sodium salt, Ethyl(2-mercaptobenzoato-(2-)-O,S) mercurate(1-) sodium (IUPAC)
phHg: phenylmercury, C_6H_5Hg

- phHg acetate: phenylmercuric acetate, acetoxyphenylmercury, acetyloxy(phenyl)mercury (IUPAC), mercuriphenyl acetate, phenomercuric acetate

Refs:
https://pubchem.ncbi.nlm.nih.gov/compound/Thimerosal,
https://echa.europa.eu/el/substance-information/-/substanceinfo/100.000.192 ,
https://pubchem.ncbi.nlm.nih.gov/compound/Phenylmercuric-acetate

3.1.1.3 Trade names and abbreviations

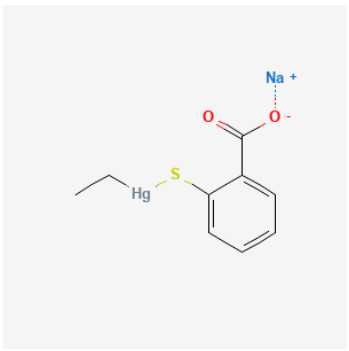
THI: Vitaseptol, Nosemack, Elicide, Aeroaid spray, Elcide 73, Elcide 75, Caswell No. 766, EPA Pesticide Chemical Code 078901 and others.
Ref.: https://pubchem.ncbi.nlm.nih.gov/compound/16684434
phHg: phHg acetate: several dozens of tradenames
Ref.: https://pubchem.ncbi.nlm.nih.gov/compound/16682730

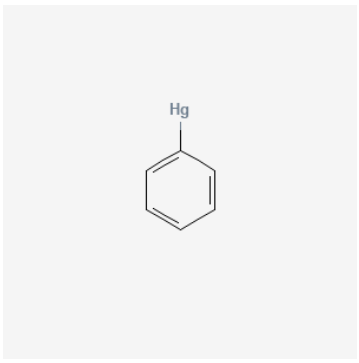
3.1.1.4 CAS / EC number

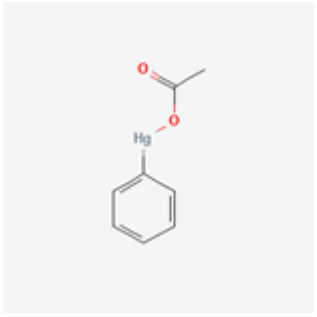
THI: 54-64-8 / 200-210-4; ethyl mercury: 16056-37-4 / --

phHg: 23172-37-4 / --
phHg acetate: 62-38-4 / 200-532-5

3.1.1.5 Structural formula

THI: 

phHg: 

phHg acetate: 

3.1.1.6 Empirical formula

THI: $C_9H_9HgO_2S.Na$; ethyl mercury: C_2H_5Hg
phHg: C_6H_5Hg

- phHg acetate: $C_8H_8HgO_2$

3.1.2 Physical form

THI: a light cream-colored crystalline powder with a slight odour
phHg: phHg acetate: Odourless, hygroscopic white or white-yellow crystalline powder

3.1.3 Molecular weight

Hg: 200.59 g/mol
THI: 404.82 g/mol
(ethyl mercury [etHg]: 229.65 g/mol)
phHg: 277.70 g/mol

- phHg acetate: 336.74 g/mol

3.1.4 Purity, composition and substance codes

THI: commercially available THI is reported to be of ≥ 97% purity (Sigma-Aldrich)
phHg: phHg acetate: commercially available THI is reported to be of 97% purity (Sigma-Aldrich, IARC monograph 58-8)

3.1.5 Impurities / accompanying contaminants

THI: none reported
phHg: phHg acetate: none reported

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3.1.6 Solubility

THI: 1000 g/L, at 20 °C (https://gestis.dguv.de/data?name=492342, last accessed 2025-03-05), 1 gr dissolves in about 8 mL of alcohol; practically insoluble in benzene and ether.
pubchem ,2008 .Ref.: Melnick *et al.*
https://pubchem.ncbi.nlm.nih.gov/compound/Thimerosal
phHg: phHg acetate: 4370 mg/L (15 °C) (ChemID, 2009); 1843 mg/L estimated with QSAR Episuite (see Appendix 2) Ref.: Anonymous 2011

17g/L in 95% ethanol, 15g/L in benzene, 48g/L in acetone (all at 15°C), 1 gr sol in 6.8 mL chloroform, 200 mL ether; Very soluble in 2-(2-ethoxyethoxy)ethanol; Soluble in acetic acid.

Ref.: PubChem

Ionization constant: 1.5 X 10^{-5}

Ref.: Parikh and Sweet 1961

3.1.7 Partition coefficient (Log Pow)

THI: log Kow = -1.88 /Estimated Ref. PubChem
phHg: phHg acetate: 0.71 (ChemID, 2009); 0.89 estimated with QSAR Episuite (see Appendix 2, ECHA 2011)

Ref.: ECHA 2011

3.1.8 Additional physical and chemical specifications

THI:
pH: 6.7 (1% aq soln at 20°C) Ref pubchem and Merck index
Melting point: 232-233ºC (decomposes)

Ref.: PubChem, Hausen 1994

Density: 2.508 g/cm^3

Ref.: Melnick *et al*. 2008

phHg:

- phHg acetate: vapour pressure: 6.00x10^{-6} mm Hg (at 20 °C) (ChemID, 2009); 1.2 mPa (35 ºC) (Tomlin, 1997); 0.016 Pa (at 25 °C) (International chemical safety cards, WHO/IPCS/ILO) (Anonymous 2011).

  Melting point: 149ºC (decomposes)

  Ref.: PubChem + Budvari 1996, p. 1257

  Density: 2.4 g/cm^3
  Ref. https://recherche.chemikalieninfo.de/public/stoff/302417?dv=18&sv=

3.1.9 Homogeneity and Stability

THI: Stable in air but it is unstable when exposed to light and when mixed with halogenides.

Ref.: Budvari 1996, Anonymous 2022

phHg: phHg acetate: Stable for at least 2 years under normal storage conditions in unopened containers. Store in a refrigerator or in a cool, dry place. Very stable in dilute acids. In the presence of alkalis, phenylmercury hydroxide is formed. Slightly volatile at room temperature.

Ref.: PubChem

3.2 TOXICOKINETICS

THI: From the abstract of Magos *et al*. 1985:
Porton Wistar male and female Rats were "given by gastric gavage five daily doses of 8.0 mg Hg/kg methyl- or ethylmercuric chloride or 9.6 mg Hg/kg ethylmercuric chloride. Three or 10 days after the last treatment day rats treated with either 8.0 or 9.6 mg Hg/kg ethylmercury had higher total or organic mercury concentrations in blood and lower concentrations in kidneys and brain than methylmercury-treated rats. In each of these tissues the inorganic mercury concentration was higher after ethyl- than after methylmercury. Weight loss relative to the expected body weight and renal damage was higher in ethylmercury-treated rats than in rats given equimolar doses of methylmercury. These effects became more severe when the dose of ethylmercury was increased by 20%. Thus, in renotoxicity the renal concentration of inorganic mercury seems to be more important than the concentration of organic or total mercury. In methylmercury-treated rats damage and inorganic mercury deposits were restricted to the P2 region of the proximal tubules, while in ethylmercury-treated rats the distribution of mercury and damage was more widespread.
There was little difference in the neurotoxicities of methylmercury and ethylmercury when effects on the dorsal root ganglia or coordination disorders were compared. Based on both criteria, an equimolar dose of ethylmercury was less neurotoxic than methylmercury, but a 20% increase in the dose of ethylmercury was enough to raise the sum of coordination disorder scores slightly and ganglion damage significantly above those in methylmercury-treated rats.
In spite of the relatively higher inorganic mercury concentration in the brain of ethylmercury- than in the brain of methylmercury-treated rats, the granular layer damage in the cerebellum was widespread only in the methylmercury-treated rats. Thus, inorganic mercury or dealkylation cannot be responsible for granular layer damage in alkylmercury intoxication. Moreover, histochemistry demonstrated no inorganic mercury deposits in the granular layer."

Ref.: Magos *et al*. 1985

The distribution of mercury as (-methyl, -ethyl and inorganic mercury) in rat tissues (brain, heart, kidney and liver) and blood following administration of THI or meHg was examined. Animals received one dose/day of Met-Hg or THI by gavage (0.5 mg Hg/kg). Blood samples were collected after 6, 12, 24, 48, 96 and 120 h of exposure. After 5 days, the animals were killed, and their tissues were collected. Total blood mercury (THg) levels were determined by ICP-MS, and methylmercury (meHg), ethylmercury (etHg) and inorganic mercury (Ino-Hg) levels were determined by speciation analysis with LC-ICP-MS. Mercury remains longer in the blood of rats treated with meHg compared to that of THI-exposed rats. Moreover, after 48 h of the THI treatment, most of the Hg found in blood was inorganic. Of the total mercury found in the brain after THI exposure, 63% was in the form of Ino-Hg, with 13.5% as etHg and 23.7% as meHg. In general, mercury in tissues and blood following THI treatment was predominantly found as Ino-Hg, but a considerable amount of etHg was also found in the liver and brain. According to the authors, the results demonstrated that the toxicokinetics of THI

is "completely different" from that of meHg. Thus, for them, meHg is not considered an appropriate reference for assessing the risk from exposure to THI-derived Hg.

Ref.: Rodrigues *et al*. 2010

According to Goldfrank's Textbook, "Limited pharmacokinetic data exist for thimerosal and ethylmercury. Once absorbed, thimerosal breaks down to form ethylmercury and thiosalicylate. Some ethylmercury further decomposes into inorganic mercury in the blood, and the remainder distributes into kidney and, to a lesser extent, brain tissue (Magos 1985, Magos 2003). Because of its longer organic chain, ethylmercury is less stable and decomposes more rapidly than methylmercury, leaving less ethylmercury available to enter kidney and brain tissue (Magos 2003). Ethylmercury crosses the blood–brain barrier by passive diffusion (...). Intracellular ethylmercury decomposes to inorganic mercury, which accumulates in kidney and brain tissues (...). The half-life of thimerosal is estimated to be about 18 days (...). Thimerosal is eliminated in the feces as inorganic mercury (...).".

Ref.: Nelson *et al*. 2011

A review of *in vitro* and *in vivo* studies that compare the toxicological parameters among etHg and other forms of mercury (predominantly meHg) had the aim to assess their relative toxicities and potential to cause cumulative insults. In the conclusion, "*in vitro* studies comparing etHg with meHg demonstrate equivalent measured outcomes for cardiovascular, neural and immune cells. However, under *in vivo* conditions, evidence indicates a distinct toxicokinetic profile between meHg and etHg, favoring a shorter blood half-life, attendant compartment distribution and the elimination of etHg compared with meHg. EtHg's toxicity profile is different from that of meHg, leading to different exposure and toxicity risks. Therefore, in real-life scenarios, a simultaneous exposure to both etHg and meHg might result in enhanced neurotoxic effects in developing mammals".

Ref.: Dorea *et al*. 2013

SCCS comment

Regarding etHg (thiomersal) toxicity, subchronic toxicity data could not be identified. Thus, despite the apparent differences in the toxicokinetic profiles of meHg and etHg, the SCCS considers meHg as a surrogate for etHg (and thus, thiomersal) toxicity, which represents a more conservative approach.

phHg: In the RAC opinion it is mentioned that "... PMA [phHg acetate] is absorbed unchanged regardless of administration route. Transportation via blood appears to be in the form of PMA. Limited data from the dog study indicates that a great proportion accumulates as PMA in the spleen in dogs. In the liver and kidneys, the PMA appears to be metabolised into inorganic mercury and accumulated. The metabolism was fairly rapid. Detectable amounts of PMA occurred for approximately 96 hours. The results indicate that rats excrete a greater proportion of PMA via urine compared with dogs. Most of the mercury was excreted via faeces."

Ref.: Anonymous 2011, section B.5.1.1

Further, "low blood concentration after administration of PMA orally or via injection indicates that PMA is rapidly removed by the tissues. In an *in vitro* study it was found that most of the PMA in human blood was bound to erythrocytes, while inorganic mercury compounds were bound to plasma. Most of the mercury in the blood was in the PMA form. In all species that were examined, PMA was found to be taken up and stored in kidney and liver. In mammals the highest levels were measured in kidneys. In these tissues PMA was mainly bound to the soluble proteins in the intercellular fluid and little was taken up by the nuclei. Only minor levels have been found in brain, heart, bone, CNS and spleen. In one study high content of mercury was found in hair and skeletal muscles (rats) and in dogs high levels of PMA were found in the spleen.

The results indicate that the excretion of PMA begins immediately after injection and diminishes nearly parallel with the concentration in blood. In all tissues, except for kidney and liver, the mercury level was markedly decreased after 96 hours post oral administration. The mercury levels in kidney and liver were at a maximum level approximately 24 hours after injection. PMA accumulated in kidney and liver. The mercury levels in liver decreased faster compared to the levels in kidney.
Short time after injection most of the PMA was recovered as organic mercury in the urine, but the level diminished rapidly. Recovered levels of inorganic mercury increased with time to a maximum at day four after a single injection. The results indicate that PMA enters the kidney and is in part rapidly excreted unchanged in the urine and in part metabolised to inorganic mercury compounds which are not as readily available for excretion. The metabolism is fairly rapid. Only a small proportion of the PMA appeared unchanged in the faeces and urine. For both oral and intravenous administration routs, the highest level of mercury was excreted via faeces. For dogs, urinary excretion was found to be lower than for rats. Faecal excretion increased rapidly with time and two days after intraperitoneal or oral administration 6-8% and 91-93% of the recovered mercury was excreted via urine and faeces, respectively."

Ref.: Anonymous 2011, section B.5.1.3

3.2.1 Dermal / percutaneous absorption

Although both dermal and inhalational absorption of organic mercury compounds are reported, precise quantitation and exclusion of concomitant absorption by ingestion are difficult to determine.

Ref.: Nelson *et al*. 2011

SCCS comment
Regarding both thiomersal and phHg salts, no publicly available scientific literature addressing dermal / percutaneous absorption was found. The SCCS has therefore applied default value of 50% absorption of the applied amount.

3.2.2 Other studies on toxicokinetics

THI: Systemically available etHg is converted to the inorganic form, contributing e.g. to a more marked renal toxicology of etHg than of meHg.

Ref.: Magos *et al*. 1985

Ethylmercury as cleaved from THI is considered its active principle. The main exposure to etHg route is parenterally through THI-containing vaccines, the target organs both the central nervous system (paresthesia, ataxia, visual and hearing loss), and the kidneys (tubular necrosis).

Ref.: Clarkson *et al*. 2003

15 female adult patients treated with 1 mL vaccine containing 0.01% THI showed a peak blood concentration of around 0.17 µg etHg/L and 1.7 µg/L total Hg, resp., one day after injection and a mean half-life of etHg of 5.6 days; after a few weeks, levels had returned to normal.

Ref.: Barregard *et al*. 2011

By contrast, the mean half-life of meHg in human blood is approximately 50 days.

ef. Rand & Caito 2019

Following single intramuscular injection of THI at 20 µg/kg, male Wistar rats exhibited Hg accumulation in the brain and liver.

Ref.: Sales *et al*. 2024

3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

To date, as regards cosmetic products, both THI and phHg derivatives are only permitted as preservatives in cosmetic eye products at a maximum concentration of 0.007 % Hg. In case of sole use of THI, this would correspond to about 0.014 % THI, and about 0.012 % etHg as cleavage product of THI. As regards phHg salts, depending on the salt, 0.007% Hg would correspond to *e.g.* 0.012 % phHg acetate.

THI: Beyond cosmetics, THI has been broadly used as preservative for vaccines dispensed in multi-dose vials, but has been largely, phased out. However, it has not completely been withdrawn, as no unequivocal evidence regarding neurodevelopmental toxicity has been identified so that a risk/benefit balance is favourable, according to the judgement of the EMEA.
Ref.: EMEA/CPMP/VEG/1194/04/Adopted.

In the USA, THI is not used in vaccines for children, except in some influenza vaccines (for which, however, a THI-free alternative is offered).
Ref.: https://www.cdc.gov/vaccine-safety/about/thimerosal.html.

In addition, THI is still in use as preservative in topical ophthalmic drug preparations, including those compounded in pharmacies; in Germany, an in-product concentration of 0.002% is recommended to achieve 3-months shelf life.
Ref.: Anonymous 2022

phHg: Independent from the use in cosmetics, following a RAC proposal for restriction, phHg acetate (along with other phHg compounds) has been restricted as entry 62 to Annex XVII to REACH. Thereby, after October 2017, the concentration of mercury shall not be greater than 0.01% w/w in manufacturing and placing on the market of mixtures. An industrial use is as catalyst in polyurethane manufacture; the major exposure of consumers due to the use of phenylmercury compounds in polyurethanes is expected to be exposure to elemental mercury and mercury compounds released during the service life of articles and exposure to mercury compounds via the environment. Indicative indoor air concentrations measured as mercury vapour between 130 and 3500 ng/m³ have been found, depending on ventilation rates, emanating from polymer flooring.
Ref.: Anonymous 2011, section B.9.3.2.2

Further characterising the risk, it was concluded that "wear and tear of surfaces may lead to increased emissions as mercury may be released from the articles and from the part of the surface which is exposed by the abrasion. In some in-door applications high levels of abrasion may be expected, e.g. gym floorings and rollers on swivel chairs. A theoretical quantitative estimation of possible air concentration of PMA in a bedroom from wheels on a swivel chair has been made, by applying the Guidance R.17. The estimated air concentrations of PMA have been compared with the derived DNEL for PMA and indicate a risk from consumer products."
Ref.: Anonymous 2011, section B.10.1.2

3.3.2 Calculation of SED/LED

$E_{dermal\ x} = C_x \times q_x \times f_{ret\ x}$

C_x = 0.007% (as mercury); bw = body weight, 60 kg as default;
other factors see **table below**.

Product type	***Calculated relative daily exposure $E_{product}$ /bw*** *(mg/kg bw/d)*
Eye shadow	**0.33**
Mascara	**0.42**
Eyeliner	**0.08**
Sum	**0.83**

Categories and estimated amounts etc. taken from Table 3B in the Notes of Guidance (12th ed.). From above sum of calculated relative daily exposure, assuming that all three relevant product types are applied, the SED is calculated:

$SED = E_{product} \times (C / 100) \times (DA_p / 100)$

$E_{product}$: above sum of calculated relative daily exposure
C : maximum permitted concentration (as Hg): 0.007%
DA_p : dermal absorption: default 50%

According to above formula,
SED = 0.83 X (0.007 / 100) X (50/100) = 0.000029 mg/kg bw/d
(0.029 µg/kg bw/d)

3.4 TOXICOLOGICAL EVALUATION

3.4.1. Irritation and corrosivity

THI: Classified in CLP with Eye Irrit. 2 (H319).
phHg: phHg acetate: Skin Corr. 1B (H314). This is not regarded as relevant with doses and type of application under consideration in this scientific advice.

3.4.2 Skin sensitisation

THI: Both a standard Buehler test and a modified Buehler test yielded a negative result with THI.

Ref.: Botham *et al*. 2005

By contrast, in most of the older experimental studies more than half of the guinea pigs could be sensitised: "Maurer 1985 Optimization test, 14/20; Novak 1983 53%; Kitamura 1990; Sun 1990; Osawa 1991 (sensitising with a DTP vaccine containing 0.01% THI), FCA-Test 4/5; Möller 1979 GPMT".

Ref.: Hausen 1994

phHg: no information on experimental sensitisation found.

Human evidence

THI: Concerning skin sensitization, THI is a well-recognized contact allergen, the only identifiable exposure having been prior vaccination in many cases.
The North American Contact Dermatitis Group found 15.3% of 391 patients aged 0 to 19 positive to thiomersal.

Ref.: Zug *et al*. 2008

In a data analysis of IVDK patch test data covering 1995-99 and 1053 patients with allergic periorbital contact dermatitis, 10.2% were found sensitized.

Ref.: Herbst *et al*. 2004

A data analysis of a subsequent period revealed a similar frequency with 9.3% positive patch test reactions in 2490 patients with periorbital dermatitis, and further 8.4% positive reactions in 22527 "control patients" who mostly still had been tested with a baseline series including THI.

Ref.: Landeck *et al*. 2014

Similarly, in North America (Canada and USA), patients patch tested between 2001 and 2016 with thiomersal (0.1% pet) showed 10.2% positive reactions with underlying atopic dermatitis and 9.1% without (not sign.).

Ref. Silverberg *et al*. 2021

Of note, in such clinical surveillance data, exposure may not necessarily have been to ophthalmic preparations or eye cosmetics, but – historically or current – to THI-preserved vaccines.
While providing clear evidence of the sensitizing potential of thiomersal, routine patch testing with THI (0.1% pet.) was mostly abandoned in lieu of use in a special test series with topical ophthalmological agents, reflecting (i) a decreasing importance as "vaccine allergen", (ii) general reservations on the actual clinical relevance of THI contact allergy as regards re-vaccination with THI-containing vaccines.

Ref.: Aberer 1991

Isolated cases of relevant contact allergy to THI-containing ophthalmic preparations, including ophthalmic drugs and contact lens cleaning solutions have been reported.

Ref.: de Groot *et al*. 1990, Herbst and Maibach 1991

THI-positive patients mostly reacted also to etHg-Cl and to a somewhat lesser extent, to thiosalicylic acid, with partial overlap, and no "isolated" reactivity to THI.

Ref.: Gonçalo *et al*. 1996

By contrast, only a small proportion of THI-sensitized also reacted to phHg borate, acetate or -nitrate.

Ref.: Aberer 1991, Uter and Lessmann 2016.

phHg: The IVDK data from 1995-99 include 892 patients with allergic periorbital dermatitis of whom 9.2% reacted positively to phHg acetate (0.05% pet.). In the subsequent study

period of 2001-2010, 884 patients with such contact dermatitis showed 8.3% positive reactions.

Ref. Landeck *et al*. 2014

However, both the 0.05% and a 0.01% concentration tested in 1151 patients simultaneously appeared less than optimal, in view of the comparative results. In conclusion, the role of phHg acetate as contact allergen is difficult to evaluate.

Ref.: Geier *et al*. 2005

SCCS comment
THI is a proven contact sensitiser, whereas evidence is equivocal for phHg acetate, owing to its irritating effect under patch-test conditions.

3.4.3 Acute toxicity

Generally, organic mercury such as meHg will result in death in doses of ≥ 0.6 mg Hg/kg bw/d following oral administration in experimental animals. With doses of 0.1-0.5 mg Hg/kg bw/d, male reproductive effects are noted, after 0.01-0.08 mg Hg/kg bw/d acute developmental effects (defects, variations) and following 0.005-0.008 mg Hg/kg bw/d neurological developmental effects.

Ref.: ATSDR 2024

SCCS comment
In view of the very low exposure to organic mercury compounds that is being considered within the scope of this scientific advice, acute toxicity is of no concern.

3.4.4 Repeated dose toxicity

THI: no data found, neither for etHg.

Organic mercury, taking meHg as most data-rich such compound, causes neurological developmental effects following intermediate, and renal, neurological, and female reproductive effects following chronic exposure to 0.01-0.08 mg Hg/kg/d in experimental animals as summarised by the ATSDR 2024 review. Intermediate exposure to levels of 0.005-0.008 mg Hg/kg/d resulted in elevated blood pressure, renal effects (clinical chemistry), behavioural effects, and male reproductive effects.

Ref.: ATSDR 2024

The studies included in the ATSDR 2024 report addressing the most sensitive endpoints of intermediate duration exposure via the oral route are described below:

Wild *et al*. (1997) studied the effects of meHg exposure on the **immune system** in the offspring of Sprague–Dawley rats at 6 and 12 weeks of age. Rats were indirectly exposed during gestation and during nursing by exposing pregnant rats to either 5 or 500 mg/L of meHg chloride (CH3HgCl) or 5 mg/L of meHg sulfide [(CH3Hg)2S] in their drinking water. Total body, splenic, and thymic weights were measured, and NK cell cytolytic activity and lymphoproliferative response to T and B cell mitogens were evaluated in the offspring. At 6 weeks of age, total body and splenic weights were significantly increased in both high- and low-dose meHg chloride-exposed groups. Rats exposed to meHg sulfide had a significant increase in thymic weight at 6 weeks of age. At 12 weeks, the total body and organ weights were not different from controls. The lymphocyte proliferative response of splenocytes to PWM was enhanced at 6 weeks in both CH3HgCl exposed groups and not affected in the (CH3Hg)2S exposed group. NK cell activity was not affected in either group at 6 weeks of age. At age 12 weeks, NK cell activity was statistically significantly decreased by 56.6% in both CH3HgCl-

exposed groups and not affected in the (CH3Hg)2S-exposed rats. The lymphocyte proliferative response of splenocytes to the B cell mitogen pokeweed remained increased in the CH3HgCl groups. The authors conclude that indirect exposure of rats (during gestation and nursing) to different forms of meHg reveals that chloride forms have prolonged predominantly enhancing effects on lymphoproliferative response of splenocytes, followed by significant depression of NK cell activity. By contrast, the bis(methylmercuric)sulfide may exhibit a preferential deposition or affinity for different targets in the immune system such as the thymus.

Ref.: Wild *et al*. 1997

Addressing **immunologic and endocrine effects**, Ortega *et al*. (1997a) studied the effects and tissue distribution of different forms of meHg compounds in rats. MeHg sulfide or meHg chloride was fed to rats at concentrations of 5 or 500 µg/L in drinking water for 8 weeks. T-cell lymphocyte proliferative response to phytohemagglutinin (PHA) and determination of tissue distribution of mercury by gas chromatography using electron capture were assayed. Four different forms of mercury compounds were employed: MeHgS-, (MeHg)2S, (MeHg)3S+, and MeHgCI. Results indicated that exposure to meHg significantly enhanced lymphocyte responsiveness in most of the exposed groups at the low concentration of 5 µg/L, with the highest proliferative response (increase) in the MeHgCI group. At 500 µg/L, a significant decrease in the lymphocyte proliferative response was observed in the (MeHg)3S+ and MeHgCI groups; conversely, the MeHgS- and (MeHg)2 S-exposed animals had a modest increase of the lymphocyte proliferative response. The largest concentrations of all four mercury forms were detected in the kidney and spleen. The levels of mercury found in kidney, spleen, liver, brain, and testis were lower in the MeHgCI group than in those exposed to (MeHg)2S and (MeHg)3S+. These data indicate that the organ distribution of mercury and immune alteration may vary according to the chemical structure of the compound. According to the authors, this observation may have important implications in humans potentially exposed to low levels of meHg present in the environment, since the immune system plays an important regulatory role in the host-defense mechanisms.

Ref.: Ortega *et al*. 1997a

Further, the effects of different meHg forms on the **immune system and the hypothalamic pituitary adrenal (HPA) axis** was assessed. The lymphocyte response to Concanavalin A (Con A) stimulation, blood levels of interleukin-6 (IL-6), adrenocorticotropic hormone (ACTH), and corticosterone in the presence of different meHg compounds was measured. Rats were exposed to meHg sulfide [(CH3Hg)2S] and meHg chloride (CH3HgCl) at concentrations of 5 or 500 µg/L in the drinking water for 8 or 16 weeks. Short-term (8 week) exposure at both, low and high doses of (CH3Hg)2S significantly enhanced lymphocyte responsiveness. MeHgCl only induced increased lymphocyte responsiveness in the low-dose exposure. Circulating levels of IL-6 after short-term exposure were increased in the MeHgCl exposed group. HPA axis activation was demonstrated by increased levels of ACTH and corticosterone levels. This response was predominant in low-dose exposed animals. Long-term (16 weeks) exposure resulted in a reduction in lymphocyte proliferation after both, low and high dose MeHgCl exposures. The (MeHg)2S exposure resulted in a 3-fold increase in the proliferative response. Levels of ACTH were elevated 3-fold in the (MeHg)2S exposed group, and no increase in corticosterone was observed in the high-dose exposed group at 8 weeks, no effect of (MeHg)2S was obsreved at 16 weeks. The MeHgCl exposed group showed an increase in ACTH and corticosterone levels at 8 weeks; this response was not observed at 16 weeks. The authors conclude that exposure to meHg compounds enhances T-cell proliferation in most of the cases, in a dose- and time-dependend fashion. Release of IL-6 also depends on the length of exposure. Early increases in circulating ACTH at 8 weeks also suggest also suggest an activation of the HPA axis. This may contribute to the production of IL-6 and surveillance of regulatory homeostatic responses against antigens that mimic stress-like responses.

Ref.: Ortega *et al*. 1997b

Wildemann *et al*. (2015) exposed normal male Wistar rats to a range of doses of inorganic mercury (as chloride; 7, 14, 29, 57, 357, 2000, 4000, 8000 mg/kg-bw/d) or meHg (as

chloride; 4, 7, 14, 29, 57, 357, 1607 mg/kg-bw/d) through the drinking water for four weeks. High-resolution ultrasound was used to measure **heart and vascular function** (carotid artery blood flow) at baseline and at the end of the exposure, while blood pressure was measured directly in the femoral artery at the end of the 4-week exposure. After 4 weeks, meHg-exposed rats had increased blood pressure at all doses despite dilated carotid arteries. Inorganic mercury did not show any significant cardiovascular effects. Based on the current study, the benchmark dose level 10% (BMDL10s) for systolic blood pressure for inorganic mercury and meHg are 1.3 and 1.0 mg/kg-bw/d, respectively. However, similar total mercury blood levels attributed to inorganic mercury or methylmercury produced strikingly different results with inorganic mercury having no observable effect on the cardiovascular system but meHg increasing systolic and pulse pressures. In conclusion, adverse cardiovascular effects cannot be predicted by total blood mercury level alone and the mercury species of exposure must be taken into account.

Ref.: Wildemann *et al*. 2015

Expanding the above study (Wildemann *et al*. 2015), Wildemann *et al*. (2016) exposed male rats (Wistar) to lead, inorganic mercury, meHg or two mixtures of all three metals for four weeks through the drinking water. The two mixture ratios were based on ratios of known reference values or environmental exposure from the literature. To investigate the potential mechanism of actions, blood pressure was measured after four weeks and compared to plasma nitrotyrosine or reduced/oxidized glutathione levels in liver as markers for oxidative stress. **Plasma renin and angiotensin II levels** were used as markers for RAS activation. Finally, **kidney function and injury** were assessed via urinary and plasma creatinine levels, creatinine clearance and urinary kidney-injury molecule (KIM-1). Inorganic mercury did not affect blood pressure or any endpoint examined. Conversely, meHg increased RAS activation along with blood pressure. Moreover, meHg in the lower concentration of 7 µg/kg bw/d was associated, as a trend, with increased angiotensin II levels, and with significantly increased plasma renin and urinary, but not blood creatinine levels.

Ref.: Wildemann *et al*. 2016

The study by Bourdineaud *et al*. (2011) examined whether a diet mimicking the fish consumption of Western populations could result in observable adverse effects in mice (age 3 weeks at start of the experiment). A vegetarian-based mouse diet was supplemented with 1.25% of lyophilized salmon flesh (SAL diet), or 1.25% of a blend of lyophilized cod, tuna, and swordfish (CTS diet). Total mercury contents were 1.15± 0.15, 2.3 ± 0.1 and 35.75 ± 0.15 ng Hg/g of food pellets for the control, SAL and CTS diets, respectively. After two months feeding, the CTS diet resulted in significant observable effects as compared to the control and SAL diets, encompassing decreased body growth, altered behavioral performance and increased anxiety level, modification of mitochondrial respiratory protein subunit concentrations in kidney and brain structures, modified gene expression patterns in kidneys, liver and muscles, and a decrease of dopamine concentrations in the hypothalamus and striatum. Of note, 1.25% of CTS flesh in the diet corresponds to an average exposure to MeHg below the WHO provisory tolerable weekly intake (PTWI) (1.6 µg MeHg/kg of body weight/week) which is exceeded by many people in Western populations, among them women of child-bearing age.

Ref.: Bourdineaud *et al*. 2011

Table 3.4.4-1: Summary of different, most sensitive toxicological endpoints examined in experimental intermediate-duration oral exposure studies with meHg (after ATSDR 2024). NOAEL/LOAEL in µg Hg/kg bw/day.

Species	Duration	NOAEL	LOAEL	Effect	Ref.
Rat	14-16 weeks premating to PND 21	--	0.3*	*Immunodevelopmental*: enhanced lympoproliferation in response to mitogens in PND 12 offspring	Wild *et al*. 1997
Rat	14-16 weeks premating to PND 21	--	0.3*	*Immunodevelopmental*: enhanced lympoproliferation in response to mitogens in PND 84 offspring	Wild *et al*. 1997
Rat	14-16 weeks premating to PND 21	--	0.6*	*Immunodevelopmental*: enhanced lympoproliferation in response to mitogens, decreased NK cell activity in PND 6-12 offspring	Wild *et al*. 1997
Rat	8 weeks	--	0.4	*Immunological*: Stimulation followed by suppression at higher doses	Ortega *et al*. 1997a, 1997b
Rat	8 weeks	--	0.4	*Endocrine*: Increase in adrenocorticotropic hormone	Ortega *et al*. 1997b
Rat	4 weeks	3.25	5.6	*Cardiovascular*: Elevated systolic blood pressure and pulse pressure	Wildeman n *et al*. 2015
Rat	4 weeks	--	6.5	*Renal*: Elevated renin and urinary creatinine	Wildeman n *et al*. 2016

PND, post-natal development day; *, derived by ATSDR 2024, not indicated in original publication.

SCCS comment

The experiments using oral exposure to meHg and addressing different toxicological endpoints as described above identified NOAEL and/or LOAEL estimates that varied to the extent summarised in Table 3.4.4-1, which shows the most sensitive result per endpoint. For immune(developmental) and hormonal effects, no NOAEL could be determined, and the LOAELs ranged between 0.3 and 0.6 µg Hg/kg bw/d. Cardiovascular and renal effects in terms of a LOAEL were noted for doses between 5 and 8 µg Hg/kg bw/day; a NOAEL could be partially derived.

As is evident from the ATSDR report (2024), methylmercury and inorganic mercury are well known to affect the immune system, leading to significant physiological alterations such as hypersensitivity and autoimmunity in both humans and experimental animals. Earlier studies have reported that methylmercury compounds can exert a stimulatory effect on

immunocompetent cells at relatively low exposure levels (below approximately 5 µg/kg bw/day). However, these studies present several important limitations, including small group sizes, unspecified compound purity, lack of randomization, and restricted dose ranges. Moreover, the endpoints investigated—such as lymphocyte proliferation—represent intermediate biomarkers rather than direct indicators of adverse effects on functioning of the immune system. In the absence of a demonstrated adverse outcome, such findings should be interpreted with caution. For these reasons, the available studies were not considered suitable for deriving a PoD.
The SCCS thus considers, as PoD, 6.5 µg Hg/kg bw/day as LOAEL for renal damage (Wildemann *et al.* 2016), corroborated by a similar LOAEL regarding cardiovascular effects (Wildemann *et al.* 2015).

phHg: phHg acetate (PMA): "Target organ for sub-chronic and chronic exposure to PMA in rats, rabbits and guineapig is the kidney. In all studies where the mercury levels were measured, mercury was found to accumulate in this tissue. In sub-acute experiments, rats administrated (ip) 10 or 20 mg PMA/kg bw/w and rabbits administrated (iv) 5 mg PMA/kg bw/w for two weeks showed no effect of the treatment. Subcutaneous administration of PMA to rats for six weeks (5.8 mg PMA/kg bw/week) did not cause any effects. Also when rabbits were exposed for ten weeks to PMA at lower dose (ip, 0.5 – 1.0 mg/kg bw/w) no effect of the treatment was reported. However, when rats were given PMA intraperitoneally for four weeks (3.5-8.75 mg PMA/kg bw/w), increased weight loss and apathy were observed. No neurological effect of PMA has been reported in other studies described in this report.
Anonymous 2011, section B.5.6.3

In the study by Fitzhugh *et al*. 1950 rats were fed on a phenylmercury acetate containing diet for two years (0, 0.1, 0.5, 2.5, 10, 40, or 160 mg of mercury in the form of phenylmercury acetate per kg diet). Chronic oral exposure of female rats to PMA from 0.5 ppm mercury (0.042 mg PMA/kg bw/d correspond to 0.29 mg PMA/kg bw/w) in the diet caused enlargement of the kidneys and moderate kidney damage (e.g., tubular dilation, atrophy, granularity, fibrosis). No differences in renal damage were observed between controls and females receiving 0.1 ppm mercury (corresponding to 0.0084 mg PMA/kg bw/d or 0.059 mg PMA/kg bw/w). At higher doses renal lesions were observed in both males and females. At all dose levels exposed animals accumulated mercury in the kidney and liver. The females seem to be more susceptible than the males. Based on these results a NOAEL of 0.1 ppm (corresponding to 0.0084 mg PMA/kg bw/day), is suggested by the EPA (EPA, 2010). The Norwegian Institute of Public Health as rapporteur of the RAC report supports this NOAEL."
Ref.: Anonymous 2011, section B.5.6.3; Fitzhugh *et al*. 1950

SCCS comment
As regards repeated dose toxicity of phHg acetate, the chronic exposure study by Fitzhugh *et al.* (1950) is apparently the only study which could support derivation of a PoD, as also noted by other agencies. Due to the fact that higher mercury levels were found in kidneys and livers of rats fed on a 0.1 ppm diet, it has been suggested that this long-term study in the rat failed to demonstrate a NOEL (www.inchem.org). However, it is unclear whether Hg accumulating in the kidney and liver following a phHg acetate diet do represent an adverse effect. Taking everything into consideration, the SCCS considers 0.1 ppm and the exposure value derived from it (see 3.5) as NOAEL.

3.4.5 Reproductive toxicity

3.4.5.1 Fertility and reproduction toxicity

THI: "Twenty-four adult male albino rats were sorted into four groups (n = 6). The first group was a control group. Rats of experimental Group 2, 3 and 4 were treated with various dosages

of thimerosal (0.5, 10, 50 mg/kg) respectively. Rats were decapitated after thirty days of trial and different parameters were analyzed. THI exposure resulted in a significant decrease in antioxidant enzyme activities including catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), glutathione reductase (GSR) and increased levels of thiobarbituric acid reactive substances (TBARS). Different doses of THI significantly decreased ($p < 0.05$) the concentration of plasma testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Additionally, Daily sperm production (DSP) and efficiency of daily sperm production were significantly reduced followed by THI exposure. Moreover, THI significantly ($p < 0.05$) decreased the primary spermatocytes, secondary spermatocytes, number of spermatogonia along with spermatids. THI induced adverse histopathological and morphological changes in testicular tissues such as decreased Leydig cells, diameter of seminiferous tubules, tunica albuginea height and epithelial height. On the other hand, the increase in tubular lumen and interstitial spaces was observed due to THI. These outcomes indicated that THI has potential reprotoxic effects in male albino rats".

Ref.: Ijaz *et al*. 2020

The study by Friedmann *et al*. (1998) used, for 19 weeks, adult male Brown Norway rats, administering meHg twice weekly at 0.8, 8.0, or 80 mg/kg. The lowest dose corresponds approximately 0.8 µg Hg/kg bw/d. Following 11 weeks of Hg treatment, males were permitted to cohabit with females. Intratesticular testosterone levels in the high-dose group were reduced by 44%, suggesting that steroidogenesis in these animals was dramatically impaired. Although sperm production was not significantly affected, numbers of sperm in the cauda epididymides of the high-dose group were reduced by 17%. Furthermore, there was a negative correlation between fertility and testicular Hg content. A dose of 8 µg Hg/kg bw/d was associated with no viable litter being produced, while with 0.8 µg Hg/kg bw/d a (non-significantly) reduced mean litter size, compared to control, was seen. According to the authors, their results raise the possibility that exposure to Hg at levels consumed by humans may result in steroidogenic impairment, reduced sperm counts, and fertility problems.

Ref.: Friedmann *et al*. 1998

phHg: phHg acetate (PMA): "Exposure [of Microtus ochrogaster in terms of a single intraperitoneal dose] to 0.125 mg/kg bw PMA at day 8 in the gestation caused significantly increased implantation loss. The results also indicated that the toxic effect of PMA increased with increased dose and decreased embryo development. No maternal toxicity was observed in animals receiving a dose of 0.06 mg [PMA]/kg bw. Taken together, the available data indicate that PMA causes adverse effect on reproduction. ... The study design of Hartke *et al*. 1976 is not in accordance with OECD test guidelines for reproduction toxicity studies."

Ref.: Hartke *et al*. 1976, Anonymous 2011, section B.5.9

SCCS comment

The available studies do not offer potential PoDs for repeated dose toxicity endpoints: Ijaz *et al.* 2020 and Hartke *et al.* 1976 employed single, relatively high-dose exposure; in the Friedmann *et al.* 1998 study, significant effects (on intratesticular testosterone levels) were seen only with the highest dose, corresponding to 80 mg/kg/d meHg, i.e., several orders of magnitude above the most sensitive endpoints listed under 3.4.4.

3.4.5.2 Developmental Toxicity

Developmental effects on the immune system have been noted with meHg in rat models with a LOAEL ranging from 0.0003 to 0.0006 Hg mg/kg bw/day, see also Table 3.4.4-1 above.

Ref.: ATSDR 2024

3.4.6 Mutagenicity / genotoxicity

THI: See 3.4.6.1.
phHg: phHg acetate (PMA): "Two *in vitro* studies showed that exposure to PMA causes SCE, and also induced high frequency of endoreduplication in cultured human lymphocytes (Lee C. *et al*., 1997; Lee C. *et al*., 1998). PMA has been found to elevate the frequency of micronuclei in the root cells of Allium (Dash S. *et al*., 1988). Human data indicates a weak alteration in the chromosome distribution in the PMA-exposed population, i.e. a possible implication of mercury in the nucleolar activity, although with extremely low exposure level. At this exposure level an increased frequency of hyperploidy was observed. Based on these data PMA may be mutagenic, but the existing data are insufficient for classification as a mutagen."

Ref.: Anonymous 2011, B.5.7.x

3.4.6.1 Mutagenicity / genotoxicity *in vitro*

Several papers have been found by the SCCS in the literature regarding the THI genotoxicity assessment *in vitro* and are summarized below.

Table 3.4.6.1-1: Summary of genotoxicity studies *in vitro* found in literature. No indication of the purity of test item, unless stated. THI, thiomersal.

Endpoint/Test system	Result	Comments by SCCS	Ref.
Micronucleus (MN)/ Chinese hamster cells (Cl-1)	**Negative** 24h exposure to 0.05, 0.1, 0.25, 0.5 µg/mL 2-5 independent experiments 2000 cells/dose The number of MN observed after THI treatments was significantly higher than control only at the highest dose (small increase: 13.7 vs 12.1) . Frequencies of CREST-positive MN after THI were not statistically different from controls	**Limited relevance.** Data are not sufficient as evidence of a positive effect since the only weak significance observed was obtained at the maximum tolerated dose. Full datasets not available for assessment.	Antoccia *et al*., 1991
Micronucleus (MN)/ Chinese hamster Luc2 lung cells	**Equivocal**	**Low relevance** of the cell type as not one recommended and equivocal results.	Lynch *et al*., 1993
Micronucleus (MN)/ cultured human diploid fibroblasts	**Negative**	**Low relevance**. Full datasets not available for assessment.	Bonatti 1992
Micronucleus (MN)/ human lymphocyte micronucleus	**Negative** both with and without S9, and treated in G1 or G2 phase 0.06 and 0.12 µg/mL tested for MNs	**Limited relevance** due to few concentrations tested. Evaluated with the human lymphocyte '*in vitro*' micronucleus test using the cytochalasin-B technique, +/-S9.	Van Hummelen and Kirsch-Volders, 1992

		An initial experiment was performed to find the maximum dose; this was the concentration where toxicity (cell death) appeared in the case TM (0.25 /µg/mL) or the maximum concentration that did not show any precipitation. Only 2 concentrations (0.06 and 0.12 µg/mL) were tested for MN.	
Micronucleus (MN)/ human lymphocytes	**Equivocal** Exposure 48 or 72h Concentrations tested: 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 0.28, 2.56, 10, 100 µg/mL Weak but significant induction of MN at concentrations between 0.01 and 0.16 µg/mL in two out of three experiments.	**High relevance**. MN using cytochalasin B for a large number of chemicals with coherent results. Interpretation as equivocal may be more suitable than positive, in view of lack of effect at higher concentrations and lack of dose-response, even below cytotoxic levels.	Migliore Nieri (1991)
Micronucleus (MN)/ isolated human lymphocytes	**Positive** Tested between 0.05–0.6 µg/mL Positive at non-toxic concentrations between 0.05–0.5 µg/mL	**High relevance** The variations in the genotoxic response to THI were seen in different donors, with different background levels, but no evidence of the involvement of the GSTM1 genotype was observed.	Westphal *et al* 2003
MN and Chromosome aberration/ human lymphocyte	**Positive** 0.5, 1, 1.5 µg/mL Highest concentration not analysed due to toxicity	**Limited relevance** Data for CA not shown in detail, 250 metaphases analysed (instead of 300 required) and not clear the use of replicates and if 2000 BNC were analysed per dose.	Mačkić-Đurović *et al*., 2022
Micronucleus (Modified micronucleus assay for detection of aneugenic effects)/ V79 cells THI (CAS No. 54-64-8) Sigma (Deisenhofen, Germany),	**Positive at high concentration** Exposure 3 h to 0.003, 0.1, 0.3 and 1 µg/mL Mitoses shaken off and seeded in new flasks. 3.5-h recovery period, and fixation MN were analyzed in 2000 .	Method not compliant with TG thus **low relevance**	Seelbach *et al*, 1993

	THI induced MN frequencies of about 12% at a concentration of 1.0g/mL. Lower concentrations gave negative results. MI was slightly increased only in the highest concentration in one experiment		
Mitotic aneuploidy /primary Chinese hamster embryonic cells	**Positive** Ten different concentrations ranging from 0.15µg/mL to 1.50 µg/mL were tested and the cells were fixed after 24-h treatment. There was a severe inhibition of mitosis >0.9 µg/mL. There was a strong inhibition of cell cycle progression as evidenced by complete lack of second division cells at concentrations above 0.60 µg/mL. There was a clear increase in the frequencies of aneuploid cells. There was an increase in the frequencies of polyploid cells	Not TG compliant **Low relevance**	Natarajan *et al*., 1993
Mutations (Ames test)/ *Salmonella typhimurium*	**Negative**	(publication not accessible)	Zeiger *et al*. 1987
Mutations (Ames test) and DNA damage/ Salmonella and E. coli DNA polymerase A assay	**Positive** with THI plus and illumination with visible light *S.typhimurmm* strains TA98, TA1538, TA1537 and TA100 E coli W3110 (polA +) and p3478 (polA-)	**Low relevance**. 4 strains of Salmonella were tested for mutagenicity in 4 strains of Salmonella and for their ability to induce repairable DNA damage in the E. coli DNA polymerase A− assay their ability to induce repairable DNA damage in the E. coli DNA polymerase A− assay. THI was capable of causing repairable DNA damage, but was active only when the plates were incubated under	Lovely *et al*. 1992

		conditions of illumination, and thus was light-induced.	
Mutations/ L5178Y mouse lymphoma (TK +/) system/ mouse lymphoma cells with and without exposure of the cells to ultraviolet A (UVA) radiation.	**Positive** Up to 3 µg/mL exposed for 1 h at 37 degrees C, and then aliquots were irradiated with UVA radiation. Slightly mutagenic and when cells were exposed to preservative and UVA radiation, the mutagenic activity of THI was enhanced.	Very toxic to cells. The concentration required to kill 50% of the cells as a result of a 1 h exposure was approx. 3 µg/mL Is mutagenic in L5178Y mouse lymphoma cells when given along with a UVA exposure. **High relevance in case of UVA** irradiation, providing evidence of photo-mutagenicity.	Withrow *et al*., 1989
Sister chromatid exchanges (SCE)/ cultured human lymphocytes THI (CAS No. 54–64–8)	**Positive** Significant induction of SCEs at concentrations 0.2 to 0.6 µg/mL both with and without S9 (100 cells were scored in blind per dose for SCEs)	The SCE is no longer included in OECD TG **Low relevance**.	Eke and Çelik 2008
Mitotic chromosome malsegregation/ Saccharomyces cerevisiae D61	**Positive**	Not compliant with TG **Low relevance**.	Albertini 1990
c-mitotic effects/ mouse bone marrow cells	**Negative**	three criteria chosen are considered as an indicative pre-screening test for the aneuploidy inducing potency: changes of the mitotic index, induction of chromatid contraction and spreading and decrease of anaphase frequencies. Not compliant with TG **Low relevance**.	Miller and Adler 1989

Data on the **gene mutation** induction by thiomersal suggests **photo-mutagenicity** of the substance, although potential mechanisms are not understood (Lovely *et al*. 1992; Withrow *et al*., 1989). In these reports, and in a report on Ames test of low relevance, negative results were observed in the absence of light (Zeiger *et al*. 1987), suggesting that in such situation no increase of gene mutations occurs. However, it is noted that the studies have limitations, are not in line with current test guidelines, and full sets of data are not available to SCCS.

Conversely, existing data on the **induction of chromosomal lesions** by thiomersal is contradictory. A recent study by Mačkić-Đurović *et al*. (2022) reported positive effects in the chromosomal aberration and micronucleus test in human lymphocytes when tested in human lymphocytes in concentrations of 0.5 µg/mL and 1 µg/mL, while toxicity at 1.5 µg/mL was reported. The data presentation and study description reveal limited relevance.

One study of high relevance used the micronucleus assay to investigate the genotoxicity in human lymphocytes after 48h or 72h exposure to 0, 0.005,0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 0.28, 2.56, 10, 100 µg/mL of thiomersal (Migliore and Nieri, 1991). A weak but significant induction of MN at concentrations between 0.01 and 0.16 µg/mL in two out of three experiments was reported, and due to the lack of effect at higher concentrations and absence of a dose-response, the SCCS considers this as an **equivocal** response.

Another study, also of high relevance used the **micronucleus** assay in human lymphocytes from several donors and investigated also if the glutathione S-transferase polymorphisms (GSTM1, GSTT1, and GSTP1) may influence the outcome of the *in vitro* micronucleus test (Westphal *et al*, 2003). It was **positive** in 14 out of 16 experiments at non-toxic concentrations between 0.05–0.5 µg/mL. In six experiments this increase was dose-dependent, at least two consecutive concentrations having significantly increased aberration frequencies. No evidence of the involvement of the GSTM1 genotype in the variations detected was observed.

Additional 5 studies of less relevance can be found in Table 3.4.6.1-1, using MN assay, and evidencing negative (2), positive (1) or equivocal results (2). Furthermore, 2 unconventional approaches to investigate mitotic effects lead to contradictory outcomes (Albertini 1990; Miller and Adler 1989) and significant induction of SCEs at concentrations 0.2 to 0.6 µg/mL was reported (Eke and Çelik 2008).

SCCS conclusion on *in vitro* genotoxicity
Several *in vitro* studies are available on scientific literature that do not allow to exclude the concern over a potential genotoxic effect of thiomersal. It is noted that most studies were not performed according to current testing guidelines, thus have limitations on the interpretation.

3.4.6.2 Mutagenicity / genotoxicity *in vivo*

Two *in vivo* studies were found, both performed before 2013, which are described in Table 3.4.6.2-1:

Table 3.4.6.2-1: Summary of genotoxicity studies *in vivo* found in literature. No indication of the purity of test item, unless stated.

Endpoint/Test system	**Result**	**Ref.**
Micronucleus and Aberrations/ Swiss CD-I male mice	Positive Induced Mn and CAs	Marrazzini *et al*.,1994
MN/male and female (102/ E1_C3H/E1) F1 mice and Swiss albino mice	Negative	Adler, *et al*. 1991

Swiss CD-I male mice were exposed to THI in one study by an international programme coordinated and partially supported by the European Community aimed at the validation of assays for the detection of aneugens (Marrazzini *et al*., 1994). A preliminary MN test, involving three animals per point, was performed to select suitable doses and sampling times in order to obtain both induction of a clear toxic effect on erythropoiesis and maximum induction of micronucleated polychromatic erythrocytes (MNPCEs). The most effective treatment was repeated in the second experiment by using six animals including three controls. Since at least 3000 polychromatic erythrocytes (PCEs) were scored per mouse, a total of more than 18 000 PCEs were analysed in six animals. THI produced a slight but significant increase in MN at a dose of 15 mg/kg at 24 h. The second experiment confirmed the results even at 48 h. Hyperploid cells did not significantly increase, while CAs reached statistical significance at

10 mg/kg 48 h. It is noted the poor presentation of the data, lack of information on exposure route, and apparently the low number of animals used in each exposure (apparently 3).

A second *in vivo* study was reported by Adler *et al*. (1991). Data was provided for 5 animals from each sex exposed once by i.p. injection to 5, 10 and 20 mg/kg, done in 2 different laboratories, and did not induce micronuclei in mouse bone marrow cells, at 24 h sampling. Although no myelosuppression was observed, systemic availability is expected from the i.p. exposure.

SCCS comments on *in vivo* genotoxicity
Contradictory results have been found in the literature regarding the potential chromosomal damage induction by THI, while no data was found regarding gene mutations *in vivo*.

SCCS overall conclusion on genotoxicity
Available data is in general of low quality, and the description of the test substance is not addressed properly to allow sufficient assessment. The SCCS is unable to conclude on the genotoxicity of thiomersal because the (i) findings were partly positive, partly negative or equivocal (regarding some genotoxicity studies) and were of (ii) low or limited reliability.

3.4.7 Carcinogenicity

THI: Possible carcinogenic effects of THI were investigated only in one study by Mason *et al*. (1971) on the chronic toxicity in Fischer 344 rats. In accordance with the intended use, THI was applied by subcutaneous injection (0.1, 0.3, and 1.0 mg/kg body weight in physiological saline, twice weekly for 12 months). Half of the animals of each experimental group were killed after 12 months and the other animals were kept for an additional 6 months. A non-significant increase of tumours was found in the high-dose THI group, therefore no carcinogenic effect was identified. However, this study does not meet the requirements of the current guidelines.
phHg: meHg compounds as a group have been categorized by IARC as Group 2B: Possibly carcinogenic to humans (https://monographs.iarc.who.int/list-of-classifications). Specifically concerning phHg acetate (PMA): no data found.

Ref.: Anonymous 2011, section B.5.8

SCCS comment
No conclusive evidence on carcinogenicity for THI is identified. The IARC classification of "meHg compounds" as a Group 2B carcinogen does not relate to phHg acetate, for which no conclusive evidence has been identified.

3.4.8 Photo-induced toxicity

THI: Two studies suggest photo-mutagenicity of THI, although potential mechanisms are not understood (Lovely *et al*. 1992; Withrow *et al*., 1989), see 3.4.6.1.

phHg: phHg acetate (PMA): no data found.

3.4.9 Human data

Human data on skin sensitisation are shown in Section 3.4.2.

3.4.10 Special investigations

THI:
The effects of meHg and etHg (the latter liberated by THI) exposure on human peripheral blood mononuclear cells (PBMCs), which are critical components of the mammalian immune system, were examined following exposure to 2.5 µM meHg or 2.5 µM etHg. PBMCs exposed to etHg showed decreased viability and size, increased granularity, reactive species production, and apoptotic indexes accompanied by an intensification of Sub-G1 and reduction in G0-G1 cell cycle phases. Preceding these effects, mitochondrial dysfunctions, namely a reduction in the electron transport system related to mitochondrial complex I, were found. In contrast, PBMCs exposed to meHg showed only reduced viability. In ICP-MS analysis, PBMCs treated with etHg accumulated Hg^{+} levels about 1.8-fold greater than meHg-exposed cells. The authors conclude these findings show that etHg is more immunotoxic to human PBMCs than meHg.

Ref.: de Souza Prestes *et al*. 2023

The formation of neutrophil extracellular traps (NETs) in response to THI and its metabolites ethyl mercury (etHg), thiosalicylic acid, and mercuric ions (Hg(2+)) was investigated. Only etHg and Hg(2+) triggered NETosis. It was independent of PKC, ERK1/2, p38, and zinc signals and not affected by the NADPH oxidase inhibitor DPI. Instead, etHg and Hg(2+) triggered NADPH oxidase-independent production of ROS, which are likely to be involved in mercurial-induced NET formation.

Ref.: Haase *et al*. 2016

An *in vitro* study examining the effect of THI and etHg, respectively, on human astrocytes identified inhibition of mitochondrial respiration leading to a drop in the steady state membrane potential, concurrently increases in the formation of superoxide, hydrogen peroxide, and Fenton/Haber-Weiss generated hydroxyl radical. These oxidants increase the levels of cellular aldehyde/ketones. Additionally, a five-fold increase in the levels of oxidant damaged mitochondrial DNA bases and increases in the levels of mtDNA nicks and blunt-ended breaks were found.

Ref.: Sharpe *et al*. 2012

SCCS comment
The relatively recent studies presented above point to immunotoxic effects and mechanisms of etHg. However, reservations concerning the use of such studies to derive a PoD as discussed in 3.4.4 apply.

3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

THI: Since for THI no point of departure can be identified, owing to the lack of suitable toxicological data specifically for etHg, a LOAEL for meHg summarized in the ATSDR 2024 review will be considered, as discussed under 3.4.4 (SCCS comment on THI).

As PoD, 6.5 µg Hg/kg bw/day as LOAEL for renal damage will be considered. Other parameters as above.

Ref.: Wildemann *et al*. 2016

MoS = PoD / SED
= 6.5 µg Hg/kg/day / (0.029 µg Hg/kg bw/d * 3)
= 74.7

phHg: Fitzhugh *et al*. (1950) studied chronic oral toxicity of phenylmercury acetate in rats, see also section 3.4.4. Rats were fed on a phenylmercury acetate containing diet for two years (0, 0.1, 0.5, 2.5, 10, 40, or 160 mg of mercury in the form of phenylmercury acetate per kg diet). The amount of as little as 0.5 ppm mercury as phenylmercury acetate resulted in kidney damage in females after 2 years; at 0.1 ppm, only "slight" renal damage could be detected in the treated female animals, and "very slight" damage in male animals, as also seen in control rats. Higher doses of 2.5 ppm and above mercury resulted in renal lesions in males and females. EPA assumes a NOEL of 0.1 ppm (www.epa.gov, 2010). They assume that rats consume about 5% of their body weight in food per day. This results in a NOEL of 0.0084 mg PMA / kg body weight /day for a chronic diet.

Ref.: Anonymous 2011

The SCCS considers 0.1 ppm and the dose derived as NOAEL, see 3.4.4. (SCCS comment on phHg). Converted to Hg, the NOAEL of 0.0084 mg PMA / kg body weight /day corresponds to 5 µgHg / kg bw /day. Since available phHg acetate data do not provide adequate quantitative information on oral absorption, a default value of 50% will be set.

MoS = PoD / SED
= 5.0 µg Hg/kg/day / (0.029 µg Hg/kg bw/d * 2)
= 86.2

3.6 DISCUSSION

Thiomersal and phenyl mercuric salts being substances known and used for a long time, much of basic toxicological research has been performed many decades ago, thus not necessarily corresponding to current guidelines. However, owing to safety concerns regarding preservation of vaccines with thiomersal (in children) as well as ingestion of meHg by food, additional scientific literature addressing some more refined endpoints such as (developmental) immune toxicity is available for etHg and especially for meHg.

Deriving a PoD from such evidence related to etHg involved using meHg as a surrogate, which appeared justified, although conservative. Using the chosen PoD (for renal toxicity) for a MoS calculation, thiomersal cannot be considered safe at the use concentration of 0.007% as Hg in eye cosmetic products. The same holds true for phHg salts. Also, the currently available evidence for or against genotoxicity of Thiomersal and phHg acetate is unclear.

In the absence of a demonstrated adverse outcome, findings related to immunotoxicity outcomes need to be interpreted with caution. For these reasons, the SCCS has considered that the available studies are not suitable for deriving a PoD.

The SCCS is not able to conclude on the genotoxicity of thiomersal in view of (i) findings which were partly positive, partly negative or equivocal (regarding some genotoxicity studies) and (ii) low or limited reliability.

Additionally considering aggregated exposure in a broader sense – not only to different eye cosmetics used concurrently, but including sources other than cosmetics – will contribute additional exposure, increasing safety concerns.

4. CONCLUSION

In light of technical and scientific progress and SCCS' expert judgement and taking under consideration in particular the various health concerns, the SCCS is requested:

(1) to identify and justify whether there are specific concerns regarding the current use of 'Thiomersal' and 'Phenylmercuric salts' as preservatives in cosmetic eye products;

Considering that the Margin of Safety based on renal toxicity as the most sensitive endpoint is less than 100, and that genotoxicity evidence is unresolved, the use of 'Thiomersal' and 'Phenylmercuric salts' as preservatives in cosmetic eye products is not considered safe at the concentration levels currently permitted in cosmetic products.

(2) to highlight if there is a potential risk for human health from the current use of 'Thiomersal' and 'Phenylmercuric salts' as preservatives in cosmetic eye products;

The potential risk for human health from use in cosmetic eye products is aggravated by additional exposure to mercuric compounds from sources other than cosmetics.

5. MINORITY OPINION

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6. REFERENCES

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7. GLOSSARY OF TERMS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 158

8. LIST OF ABBREVIATIONS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 158

9. Annex I: Physicochemical characteristics of further phenylmercuric compounds

Chemical names

- **phHg benzoate**: Phenylmercury benzoate, phenylmercuric benzoate, (Benzoato-O)phenylmercury, benzoyloxy(phenyl)mercury (IUPAC)
- **phHg borate**: Phenylmercuric borate, boronooxy(phenyl)mercury (IUPAC), phenylmercury borate, dihydrogen [orthoborato(3-)-O]phenylmercurate(2-)
- Di(phHg) borate: [μ-[orthoborato(2-)-O:O']]diphenyldimercury, [hydroxy(phenylmercuriooxy)boranyl]oxy-phenylmercury (IUPAC)
- **phHgCl**: Phenylmercuric chloride, phenylmercury chloride, Phenyl chloromercury, mercury chlorophenyl-, (Chloromercuri)benzene, chloro(phenyl)mercury (IUPAC)
- **phHgBr**: Phenylmercuric bromide, phenylmercury bromide, mercury bromophenyl-, (bromomercuri)benzene, bromo(phenyl)mercury (IUPAC)
- **phHgOH**: Phenylmercuric hydroxide, phenylmercury hydroxide, phenylmercurol, hydroxyphenylmercury, phenyl hydroxymercury, mercury hydroxyphenyl-, phenylmercury(II) hydroxide (IUPAC)

Mixture: ((phenylmercurio)oxy)boronic acid, equimolecular compound of phenylmercury borate and phenylmercuric hydroxide, bis(phenylmercuryylium) dihydrogen borate hydroxide, dihydrogen borate;phenylmercury(1+);hydroxide (IUPAC)

, https://echa.europa.eu/el/substance-information/-/substanceinfo/100.000.484, https://pubchem.ncbi.nlm.nih.gov/compound/Phenylmercury-benzoate, https://echa.europa.eu/el/substance-information/-/substanceinfo/100.002.120 , https://pubchem.ncbi.nlm.nih.gov/compound/Phenylmercury%20borate , https://echa.europa.eu/el/substance-information/-/substanceinfo/100.002.790, https://pubchem.ncbi.nlm.nih.gov/compound/16683655, https://echa.europa.eu/el/substance-information/-/substanceinfo/100.240.860, https://pubchem.ncbi.nlm.nih.gov/compound/Phenylmercuric%20borate https://pubchem.ncbi.nlm.nih.gov/compound/Phenylmercuric-chloride https://pubchem.ncbi.nlm.nih.gov/compound/14509 https://echa.europa.eu/el/substance-information/-/substanceinfo/100.002.606

Trade names and abbreviations

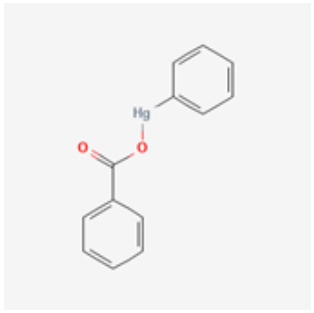
- phHg benzoate: --
- phHg borate: Famosept, Fenosept, Merfen, Metasol BT, Ryfen, Spidox
- phHgCl: Agrenal, Agronal
- phHgBr:
- PhHgOH: Mersolite 1

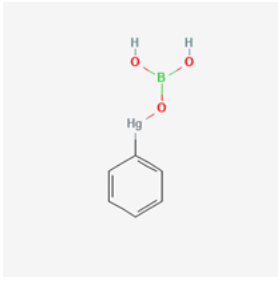
Ref: https://pubchem.ncbi.nlm.nih.gov/compound/Phenylmercury%20borate https://pubchem.ncbi.nlm.nih.gov/compound/Phenylmercuric-chloride https://echa.europa.eu/el/substance-information/-/substanceinfo/100.002.606

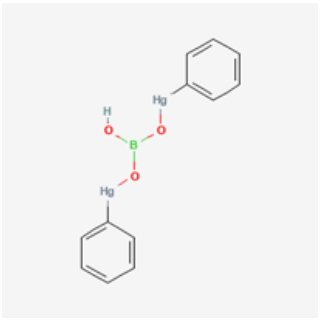
CAS / EC number

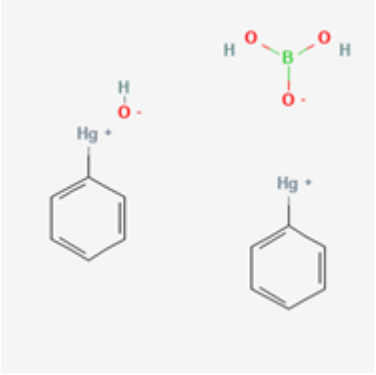
phHg benzoate: 94-43-9 / 202-331-8
phHg borate (phenylmercury borate): 102-98-7 / 203-068-1
Di(phHg) borate: [μ-[orthoborato(2-)-O:O']]diphenyldimercury: 6273-99-0 / 228-465-7
Mixture of phenylmercuric hydroxide and phenylmercuric orthoborate: 8017-88-7 / --
phHgCl: 100-56-1 / 202-865-1
phHgBr: 1192-89-8 / 214-760-8
phHgOH: 100-57-2 / 202-866-7

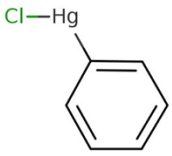
Structural formula

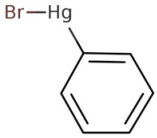
phHg benzoate: 

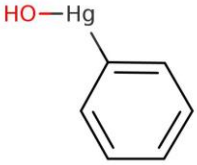
phHg borate: 

di(phHg) borate: 

Mixture: 

phHgCl 

phHgBr 



phHgOH

Empirical formula

- phHg benzoate: $C_{13}H_{10}HgO_2$
- phHg borate: $C_6H_7BHgO_3$; SMILES: B(O)(O)[O-].C1=CC=C(C=C1)[Hg+].C1=CC=C(C=C1)[Hg+].[OH-] (see https://pubchem.ncbi.nlm.nih.gov/compound/53384581)
- Di(phHg) borate: $C_{12}H_{11}BHg_2O_3$
- Mixture of phHg hydroxide and phHg orthoborate: $C_{12}H_{13}BHg_2O_4$
- phHgCl: C_6H_5ClHg
- phHgBr: C_6H_5BrHg
- phHgOH: C_6H_6HgO

3.1.2 Physical form

phHg benzoate:
phHg borate:
Di(phHg) borate:
phHgCl: White solid
phHgBr:
phHgOH:

Molecular weight

phHg benzoate: 398.81 g/mol
phHg borate: 338.519 g/mol
Di(phHg) borate: 615.21 g/mol
Mixture of phHg hydroxide and phHg orthoborate: 633.23 g/mol
phHgCl: 313.15 g/mol
phHgBr: 357.60 g/mol
phHgOH: 294.70

Solubility

- phHg benzoate: 30.96 mg/L in water at 25 °C (est) Ref.: Ref.: https://www.thegoodscentscompany.com/data/rw1302271.html
- phHg borate:
- Mixture of phHg hydroxide and phHg orthoborate: 54.1 mg/mL in water (ALOGPS) Ref.: https://go.drugbank.com/drugs/DB13834
- phHgCl: Soluble in about 20,000 parts cold water; slightly soluble in hot alcohol; soluble in benzene, ether, pyridine

phHBr:Partition coefficient (Log Pow)

phHg benzoate: 0.28 (ALOGPS) 0.9 (Chemaxon)

- phHg borate:
- Mixture of phHg hydroxide and phHg orthoborate: 0.28 (ALOGPS) 0.9 (Chemaxon) Ref.: https://go.drugbank.com/drugs/DB13834
- phHgCl: 1.78
- phHBrl:

Additional physical and chemical specifications

- phHg benzoate: Melting point: 94º C
- Ref.: https://www.chemicalbook.com/ChemicalProductProperty_EN_CB8141019.htm

Boiling Point: 249.3°C at 760 mmHg
Flash Point: 111.4°C
Vapor Pressure: 0.0122mmHg at 25°C
Ref.: https://www.lookchem.com/casno94-43-9.html

- phHg borate: no data found
- Di(phHg) borate pKa: 5.06 (predicted)

Ref.: https://comptox.epa.gov/dashboard/chemical/properties/DTXSID80897334
phHgCl: Melting point: 250-252 °C
Vapour pressure: 0.02 mmHg
Ref.: https://pubchem.ncbi.nlm.nih.gov/compound/Phenylmercuric-chloride

- phHgBr:
- PhHgOH: dissociation constant K_b = 1.3 x 10^{-10}

Ref. S. S. Parikh and Thomas R. Sweet. The Journal of Physical Chemistry 1961 65 (10), 1909-1911 DOI: 10.1021/j100827a5
