



## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### THIOMERSAL AND TIMERFONATE

#### SUMMARY REPORT

1. Thiomersal (INN);  $C_9H_9HgNaO_2S$ ; molecular weight 404.84; Ethyl [2-mercaptobenzoato(2-)-O,S]-mercurate(1-)-sodium (CAS). (Synonyms: merfamin, merseptyl, merthiolate, mertorgan, merzonin, thimerosal, thiomersalate, ethylmercurithio-salicylic acid).

The substance is soluble in water (20° C 1000 g/l) and ethanol (125 g/l) and practically insoluble in organic solvents such as ether and benzene. The thiomersal molecule contains 49.5% of mercury.

Timerfonate (INN);  $C_8H_9HgNaO_3S_2$ ; molecular weight 440.89; ethyl (4-mercaptobenzenesulfonato- $S^4$ ) mercury sodium salt (CAS). (Synonyms: thiocid, sulfo-merthiolate). The substance has a good solubility in water and is slightly soluble in ethanol. The mercury content is 45.5%.

2. Thiomersal and timerfonate are organomercuric compounds used as preservatives in vaccines, antigens and immunoglobulins intended for use in humans and animals. At present these substances seem to best fulfil the requirements for efficacious preservatives used in inactivated vaccines because they do not react with antigen and are effective at very low concentrations. According to the recent survey, 73% of inactivated multidose vaccines for food-producing animals marketed in European Union contain thiomersal as preservative. In addition the substances have a limited use in veterinary preparations for injection.

Vaccines (subcutaneous or intramuscular application) for pigs, cattle, horses and poultry contain between 0.04 and 0.1 mg thiomersal/ml. The total dose of thiomersal injected is approximately 0.1-2  $\mu\text{g}/\text{kg}$  bw (corresponding to 0.05-1  $\mu\text{g}$  Hg/kg bw) in cattle, horse and pig and 30  $\mu\text{g}/\text{kg}$  bw (corresponding to 15  $\mu\text{g}$  Hg/kg bw) in poultry. For timerfonate the doses per injection are about one order of magnitude lower resulting in 0.01-0.2  $\mu\text{g}/\text{kg}$  bw (corresponding to 0.005-0.1  $\mu\text{g}$  Hg/kg bw).

Thiomersal and timerfonate have an antimicrobial (bacteriostatic) effect which is related to inhibition of bacterial essential enzyme activity but no specific data on the mode of action are available. The antiseptic activity of the formulations is attributed to the partial ionisation of the compounds and to the fact that thiomersal and timerfonate provide a reservoir of ethylmercury.

3. No information on absorption after oral administration of thiomersal or timerfonate is available. No studies concerning metabolism of thiomersal or timerfonate *in vivo* were presented. The information available from the published literature indicates that in the acidic environment of the stomach thiomersal and timerfonate will be cleaved into thiosalicylic acid and ethylmercury and into 4-thiophenyl-sulfonic acid and ethylmercury respectively. Experiments also show that ethylmercury in the tissues is partially converted to the inorganic forms of the element.

Generally organomercury compounds are readily absorbed in the gastrointestinal tract, e.g. between 90 and 100 % of methylmercury is absorbed. There is some evidence from acute poisonings in man indicating that ethylmercury is also effectively absorbed after ingestion. In comparison only less than 10% of the inorganic mercury is estimated to be absorbed from the

gastrointestinal tract. (International Program on Chemicals Safety (IPCS), Environmental Health Criteria 101, WHO 1990).

4. Distribution of thiomersal was examined in squirrel monkeys (400-900 g bw) after dosing different concentrations of thiomersal intranasally for six months in daily doses of 2.2 µg (low dose) and 12.0 µg thiomersal (high dose). The tissue concentrations were expressed as total and inorganic mercury. The inorganic mercury in different organs was 37-91% of the total mercury concentration. Only the high dose group showed a clear increase in mercury as compared to the controls.

The increase was seen in all tissues except blood with highest levels of the element detected in the kidney, followed by the liver and brain. The ratio between the mercury concentration in tissue from high level administration and control group animals was about 3 for brain, 10 for liver, 8 for kidney and 5 for muscle.

A similar distribution pattern was seen after a single subcutaneous and/or ophthalmic application of <sup>203</sup>Hg-labelled thiomersal (corresponding to 0.55 mg Hg/kg bw) to pregnant rabbits. Two to six hours after the application of thiomersal the highest concentrations of mercury (measured as radioactivity) were found in kidney and liver for both the treated animals and their offspring.

A comparison of oral administration, subcutaneous injection and inhalation of <sup>203</sup>Hg-labelled ethylmercuric chloride in rat showed no substantial differences in the distribution pattern of <sup>203</sup>Hg mercury in the tissues. The levels in the blood and liver decreased with time while in the kidney the residues mostly in form of inorganic mercury persisted even 14 days after the exposure.

5. No information on excretion of thiomersal and timerfonate was provided. However it is known that inorganic mercury is mainly excreted via the kidney while organic mercury compounds are mainly excreted through the faeces. For methylmercury the existence of an enterohepatic cycle has been shown. Half-lives of organic mercury compounds vary extensively among species. Initial half-lives of ethyl mercury were 4.4 days in mice and 7 to 10 days in rats of different ages. Other reported values for organic mercurials range from 8 days in mouse to nearly 1000 days in some species of fish. Since ethylmercury is converted more rapidly into inorganic mercury than methylmercury (IPCS Environmental Health Criteria 1, WHO, 1976), it is likely to be excreted faster as well. The biological half-life of methylmercury in man is about 70 days. High levels of mercury directly correlating with blood concentrations can be detected in hair. There is also transfer of mercury to mothers' milk after the exposure to methylmercury.
6. Acute oral toxicity of thiomersal was tested in mice and an LD<sub>50</sub> value of 91 mg/kg bw was reported. An oral LD<sub>50</sub> value of 73 mg/kg bw was reported for timerfonate. LD<sub>50</sub> (subcutaneous) for thiomersal in rats was 98 mg/kg bw and LD<sub>50</sub> (subcutaneous) for timerfonate in mice was 32 mg/kg bw. Like other mercury-containing compounds, thiomersal and timerfonate show a relatively high acute mammalian toxicity.
7. No acceptable documentation of the subchronic toxicity of thiomersal or timerfonate was presented. Numerous studies have shown that chronic exposure to inorganic and organic forms of mercury leads to renal damage and neurological dysfunction. The principal target tissue for methylmercury in animal and man is the nervous system. Central nervous system under development (foetus) is especially sensitive to neurological disturbances. Concerning renal toxicity, the kidney concentration of inorganic mercury seems to be more important than the concentration of organic or total mercury. However, no critical renal concentration of mercury could so far be established in laboratory animals or in man.
8. There are no investigations concerning the acute or chronic toxicity of thiomersal or timerfonate in farm animals. Both substances have been used as preservatives in injectable solutions for animals as well as for man for many decades with rarely observed side-effects.
9. No studies concerning the effects of thiomersal or timerfonate on reproduction were submitted. However, methylmercury was reported to affect spermatogenesis in mice (1 mg Hg/kg bw) and treatment (3 months) with daily oral doses of methylmercuric hydroxide (0.05-0.09 mg/kg bw) resulted in an increased frequency of reproductive failure (non-conception, abortion) in non-human primates.

10. One teratology study with thiomersal tested in rats and rabbits was presented. The dosage levels and number of animals per dosage group is not in full accordance with current requirements. Thiomersal was administered (intraperitoneal estimated daily doses about 7 and 70 mg/kg bw) to two groups of 10 female rats daily on days 6 through 18 of gestation. In another experiment seven pregnant rabbits were given a 2 % thiomersal solution to both eyes on days 6 through 18 of gestation. No malformations due to the thiomersal treatment were seen in any of the foetuses of rats or rabbits. However, an increased number of dead and aborted foetuses (rats and rabbits) as well as an increased number of absorptions (rats) was reported for the treated groups. In comparison, experimental studies show that methylmercury is foetotoxic in mice (single dose of 2.5-7.5 mg/kg), teratogenic in rats and adversely affects the behaviour of monkey offspring (0.05-0.07 mg/kg per day before and during pregnancy) (ICPS, Environmental Health Criteria 101, WHO 1990).
11. The mutagenic potential of thiomersal was investigated in the Ames test, *E. coli* DNA polymerase A<sup>-</sup> assay, mouse lymphoma assay, in micronucleus tests in mice as well as an *in vitro* test in human lymphocytes. Reported results for the first three tests seem not to be very reliable. Observed effects were weak and probably not significant indicating that the substance is not gene mutagenic. Induction of genotoxicity under illumination was seen in DNA polymerase assay. Micronucleus tests provided somewhat contradictory results with induction of micronucleus in one test system but negative results in other two tests. Thiomersal induced spindle effect and aneuploidy in *in vitro* culture of human lymphocytes but no threshold could be established. The findings are in accordance with the effects of thiomersal seen in other test systems. Similar effects in genotoxic tests are also shown for other mercury compounds.
12. The carcinogenicity of thiomersal was evaluated in rats. Animals of both sexes were injected (subcutaneously) with 0.03, 0.1, 0.3 and 1.0 mg thiomersal/kg bw twice weekly for 52 weeks. Animal numbers per group were 80, 60, 40 and 20 from high to low dose. Two negative controls and one positive control were used. Animals were autopsied either 12 or 18 months after the start of treatment. Four injection site related tumours occurred in the animals given the highest dose. No other treatment-related neoplastic findings were observed. A high dose-related incidence of bronchopneumonia was reported. The results were only presented in a brief form with no original protocols available and no conclusion about the carcinogenic potential of this substance can be drawn from this study.
13. Sensitizing effects comparable to that of organic mercury compounds have been shown after the injection of thiomersal to desensitized laboratory animals. Thiomersal induced cell-mediated immunity in human studies via ocular, dermal exposure or by injection at much higher rates as compared to other disinfectants such as phenol and chlorhexidine. Isolated cases of hypersensitivity reactions in man presumably related to ethylmercury, were reported in connection with the administration of vaccines which contained thiomersal.
14. There was no information available dealing with residues of thiomersal or timerfonate in food-producing animals. It is likely that residues of thiomersal and timerfonate consist of thiosalicylic acid and 4-thiophenylsulphonic acid respectively, in addition to ethylmercury and inorganic mercury. Thiosalicylic acid and 4-thiophenylsulphonic acid are polar compounds which can easily be transformed to conjugates and these conjugates will be rapidly excreted.

Based on the doses used, the total mercury levels even a short time after the treatment of animals with thiomersal-containing vaccines can be calculated to be no higher than 0.0003-0.010 mg in muscle and other edible tissues. This would lead to an estimated highest total intake of less than or equal to 0.010 mg Hg/day (presumably in equal part inorganic and organic mercury). The exposure is in the range of reported national average daily intakes of mercury from the diet. Depending on their fish consumption, which is the single most important source of methylmercury, certain groups of consumers can be expected to have much higher intake. Similarly individuals from locally contaminated areas can be exposed to higher dietary levels of mercury.

According to Commission Decision 93/351/EEC of 19 May 1993, determining analysis methods, sampling plans and maximum limits for mercury in fishery products, the mean total mercury content of the edible parts of fishery products is restricted in general to 0.5 mg/kg of fresh weight, allowing for 20 specified species an average limit of the edible parts of 1 mg/kg of fresh weight.

No data on the mercury concentration at the injection site are available but under the worst case scenario the total amount which can theoretically be ingested from the injection site (cattle) can be calculated to be 0.5 mg mercury.

15. Almost all evaluated data are based on published literature and most of the studies were reported between 1970 and 1985. None of the studies complies with GLP standards.

There are no relevant studies for the evaluation of a NOEL for thiomersal or timerfonate. Therefore no ADI for the compounds can be proposed. It seems obvious that thiomersal and timerfonate are mainly active due to release of ethylmercury and inorganic mercury and may contribute to the total mercury body burden in consumers. Consequently, the provisional tolerable weekly intake (PTWI) of the FAO/WHO (IPCS, Environmental Health Criteria 1, WHO, 1976) for mercury can also be employed for the two preservatives. The provisional tolerable weekly intake for total mercury is 0.3 mg per person (bodyweight 60 kg).

This should be compared to the highest estimated single intake of less or equal to 0.010 mg Hg/day ingested via edible tissues from the animals treated with the thiomersal/timerfonate containing preparations.

There is virtually no information on the acute toxic effect level of dietary mercury in man but the data available (IPCS, Environmental Health Criteria 1, WHO, 1976) indicate a threshold for neurologic effects (paresthesia) in adults to be in the range of 0.5 mg/kg bw corresponding to 30 mg mercury for 60 kg person. With the application of a safety factor of 5 in order to provide for a presumed higher sensitivity of the foetus (new born) the threshold level for the acute toxic dose level would be 0.1 mg Hg/kg bw corresponding to 6 mg/person (pregnant woman). In comparison the amount of mercury which could be theoretically ingested from the injection site can be estimated to 0.5 mg of mercury.

In conclusion the contribution of mercury residues expected to be ingested in foodstuffs from animals given thiomersal or timerfonate to normal daily dietary intake of mercury is low. Compared to the average long-term dietary intake of the metal, which is the most relevant from the toxicological point of view, the contribution to body burden of mercury from thiomersal and timerfonate is negligible. The use of thiomersal and timerfonate as preservatives in vaccines can therefore be regarded as without appreciable risk to consumer health.

## **Conclusions and recommendation**

Having considered the criteria laid down by the Committee for the inclusion of substances in Annex II of Council Regulation (EEC) No 2377/90 and in particular that:

- animals are unlikely to be sent for slaughter immediately after treatment;
- the use of thiomersal and timerfonate as preservatives will add negligible amounts to the long-term exposure of the consumers to mercury from other dietary sources;
- it appears unlikely that the use of thiomersal and timerfonate in veterinary medicinal products as preservatives represents any significant risk to the consumer;

the Committee considers that there is no need to establish an MRL for thiomersal and timerfonate and recommends their inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Thiomersal	All food producing species	For use only as preservatives in multidose vaccines at a concentration not exceeding 0.02%
Timerfonate	All food producing species	For use only as preservatives in multidose vaccines at a concentration not exceeding 0.02%