

**External Letter Peer Review of a Report by
Midwest Research Institute**

**Revised Final Report: Toxicity of Thallium (I)
Sulfate (CAS No. 7446-18-6) in Sprague-Dawley
Rats, Volume Two: Subchronic (90-day) Study,
July 1988**

**Compilation of Reviewer Comments and
Responses to Charge Questions**

**Prepared for
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Office of Science and Technology
Office of Water
U.S. Environmental Protection Agency**

**Prepared by
Oak Ridge National Laboratories**

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Composite Comments on the MRI, 1988 Thallium Report

Charge for External Peer Reviewers

The U.S. Environmental Protection Agency's (EPA) National Center for Environmental Assessment (NCEA) is developing a "Toxicological Review of Thallium Salt Compounds." The Midwest Research Institute (MRI, 1988) Final Report is a proposed principal study, but has not undergone a formal peer review. It is important that selected outside experts evaluate the accuracy of the experimental procedures, results, and interpretation and discussion of the findings presented in this investigational report.

Male and female Sprague-Dawley rats (45 days old, 20/sex/group) were administered 0 (untreated and vehicle controls), 0.01, 0.05, or 0.25 mg/kg-day of an aqueous solution of thallium (I) sulfate (approximately 0, 0.008, 0.04, or 0.20 mg Tl/kg-day) by gavage for 90 days (U.S. EPA, 1986c). Endpoints evaluated were body and organ weights, food consumption rates, hematologic and clinical chemistry parameters, histopathology, neuropathology, and observations from neurotoxicologic and ophthalmologic examinations. There were no statistically significant differences in body weight, food consumption, or absolute and relative organ weights among control groups and groups receiving thallium (I) sulfate. Ophthalmology examinations did not indicate any treatment-related effects. Per the study authors, histopathology did not reveal any treatment-related effects.

Lacrimation (secretion of tears) and exophthalmos (abnormal protrusion of the eyeball) were observed at higher incidences in the treated rats compared with both controls. The incidence of lacrimation in males (M) and females (F) was as follows: untreated control C1/20 (M), 7/20 (F); vehicle control C6/20 (M), 6/20 (F); 0.008 mg Tl/kg-day C19/20 (M), 20/20 (F); and 0.04 and 0.2 mg Tl/kg-day C20/20 (M and F). The incidence of exophthalmos was as follows: untreated control C1/20 (M), 5/20 (F); vehicle control C5/20 (M), 6/20 (F); 0.008 mg Tl/kg-day C12/20 (M), 19/20 (F); and 0.04 and 0.2 mg Tl/kg-day C20/20 (M and F). Ophthalmic examination and gross and histopathological examination of the eyes, however, revealed no treatment-related abnormalities.

Subtle but statistically significant changes were observed in several blood chemistry parameters that the investigators considered probably treatment related. Specifically, dose-related increases in serum glutamic oxaloacetic transferase (SGOT, now termed AST), lactate dehydrogenase (LDH), and sodium levels and decreases in blood sugar levels were detected in male and female rats after 30 and 90 days of exposure. Reported values for the selected blood chemistry parameters are summarized in Table 3. Other changes in blood chemistry parameters were less consistent across species, dose groups, and exposure durations.

At 90 days, changes in AST, LDH, sodium, and blood sugar levels in dosed male and female rats were no greater than 31%, 38%, 4%, and 82%, respectively, of the vehicle control group values. The investigators observed that the increases in AST and LDH levels could indicate a possible effect of treatment on cardiac function, that increases in LDH coupled with subtle changes in electrolytes could indicate an effect on renal function, and that, in rare instances, a decrease in blood sugar coupled with an increase in sodium occurs as a defense

mechanism for maintaining cellular integrity. The investigators concluded that none of the changes observed in the blood chemistries of males or females during the study were of sufficient magnitude to significantly affect the health status of the animals. Further, histopathological evaluation did not confirm any cellular damage suggested by the clinical chemistry findings.

The only treatment-related finding reported at gross necropsy was the incidence of alopecia, which was noted particularly in female rats at the highest dose (see Table 4). Most instances of alopecia in females were accredited to cyclic patterns of hair growth. Of the twelve high-dose females with alopecia, 5 cases were attributed to self-barbering behavior and 5 to sporadic occurrences related to normal hair cycling patterns. Two animals had unexplained moderate to severe alopecia that was associated with atrophy of the hair follicle. However, the authors noted that the status of hair follicles in rodents is greatly affected by the cyclic pattern of hair growth as well as the location of the hair on the body. Consequently, the authors considered these findings to be biologically insignificant. Therefore, the study authors chose the highest dose, 0.25 mg/kg-day thallium (I) sulfate (0.20 mg Tl/kg-day), as the no-observed-adverse-effect level (NOAEL).

CHARGE QUESTIONS:

1. Based on your knowledge of toxicological protocols, please comment on the experimental procedures conducted in this investigation. Do you see any significant issues with the test system or article, oral exposure procedures, observations recorded, terminal procedures, statistics, and quality assurance?

Dr. Davidson Comments

The experimental procedure complies with most of the parameters in the current toxicity testing guidelines (OPPTS 870.3100). According to the report, the study was conducted in compliance with EPA TSCA guidelines in effect during the time the study was conducted. A few requirements in the current testing guidelines were not included in the study; urine was not collected and analyzed, the thymus was not weighed, and the nose, pharynx, larynx, and seminal vesicles were not examined microscopically. However, based on the results of the study and the target organs identified in the 14-day study, these deficiencies do not impact on the interpretation of the results.

The major issue in the 90-day study was dose selection. In the 14-day study, the investigators did not adequately describe the basis for selecting the doses for the 90-day study. In the 14-day study, the investigators noted that 2.5 mg/kg/day caused a transient decrease in weight gain and only one rat had microscopic alterations in the hair follicles; nevertheless, a 10-fold lower dose was selected as the high dose for the 90-day study. If the investigators discounted alopecia, lacrimation, and exophthalmos as toxicologically significant in the 14-day study, the high-dose should have been set at 1.0 or 2.5 mg/kg/day.

Dr. Talmage Comments

The study was conducted according to EPA TSCA GLP guidelines in place at the time. The reviewer found no shortcomings in the study protocol and conduct. Endpoints related to known toxic effects of thallium, including those observed in the range-finding study, were appropriately monitored. These include clinical/neurotoxicological observations including skin reddening, hair loss, abnormal behavior, and stool and urine abnormalities; histological examination of major organs; and neuropathology (brain, spinal cord, and peripheral nerves).

Dr. Koller Comments

The experiment used a standard, universally accepted experimental design to adequately evaluate the toxic effects of thallium in a 90-day study. The test agent was analyzed for purity, prepared weekly for dosing with the prepared solutions being analyzed, sufficient numbers (20/group) of male and female Sprague-Dawley rats were gavaged daily with respective dosages, naive and vehicle control groups were included, animals were cared and housed according to NIH guidelines, animals were observed daily for clinical signs, ophthalmic examinations were performed, blood was collected for hematology and clinical chemistry's, rats were weighed weekly with organ/body weight ratios recorded at necropsy, histopathology was performed on numerous organs/tissues, and the rats were evaluated for neurological effects. The rats were euthanized in accordance with regulatory standards, data were statistically analyzed and the experiments were conducted using Good Laboratory Practices and Quality Assurance. There were basically no deficiencies (see number 5 below) in the experimental design in which to answer the overall objective of this study; i.e., to determine the no-observed-effect-level and toxic effects associated with repeated exposure to thallium (I) sulfate for 90-days.

2. Are there endpoints that should have been monitored that were not part of the investigation?

Dr. Davidson Comments

Exophthalmos was observed in the range-finding study; this observation could be a sign of hyperthyroidism. A test of thyroid function (measurement of thyroid hormones) should have been conducted in the 90-day study to confirm or rule out a thyroid involvement. Exophthalmos, lacrimation, and miosis taken together suggest a neurological effect. Therefore, a definitive neurological evaluation should have been considered for the 90-day study.

Dr. Talmage Comments

The U.S. EPA functional observational battery (FOB) was not part of a neurotoxicological protocol at the time of this study, but might have been helpful in identifying subtle neurological effects. The investigators did monitor many of the neurological endpoints contained in the present-day FOB. Some sort of activity endpoint such as motor activity or time spent barbering would have been useful. Changes in motor activity might indicate a level of "discomfort" in the animals.

Dr. Koller Comments

Toxic endpoints known to result from exposures to thallium and those identified in the 14-day range finding study were evaluated in the 90-day study. In addition, a battery of hematological and clinical chemistry parameters were measured as well as histopathology performed on three-dozen organs and/or tissues. Thus, the endpoints measured in this experiment were basically sufficient to determine the toxicity of thallium (see numbers 3, 5 & 6 below). Furthermore, extra steps were taken in an attempt to diagnose an unexpected ear lesion that occurred in all groups of rats in this study.

3. Please comment on the strength, credibility, and relevance of the toxicological results. Were the results of the individual rodents correctly interpreted and summarized?

Dr. Davidson Comments

Clinical signs regarding the skin/coat and eyes showed strong dose-response relationships, although the intensity of the observation was not strong in all cases. Serum enzyme activities [SGOT (AST) and LDH] increased by less than twofold compared with the control values and the individual data showed a definite increase in activity of individual animals at 30 days in both sexes administered the mid- and high-dose. Some of the electrolytes showed increases as well. Almost all clinical chemistry value for individual animals were lower at 90 days than at 30 days, including untreated and vehicle controls. No obvious outliers were observed. No gross or histopathological lesions were found that correlated with the changes in serum chemistry values suggesting that the changes were not the result of pathological lesions and, therefore, not toxicologically significant.

Selective examination of the individual data for clinical observations and gross and histopathological changes showed some discrepancies. The reviewer examined the data for females in the high-dose group as a spot check. Alopecia was observed in eight females at study termination but was recorded in only four animals in the individual data tables for gross lesions. However, the summary table of gross lesions listed five animals with alopecia. The gross lesions omitted from the individual animal tables included a grade 1 lesion in one rat, a grade 2 lesion one rat, and grade 5 lesions in two rats. Exophthalmos was observed in 11 high-dose females during clinical observation at study termination, but was not recorded during gross examination.

Dr. Talmage Comments

Gross and microscopic examinations of tissues were thoroughly conducted. The data were well summarized. The results of the toxicological observations of the individual rodents appear to be correctly summarized. Obviously the investigators were intent on monitoring and identifying the known effects of thallium on the skin and hair. To that end, barbering, alopecia, skin lesions, lacrimation, and exophthalmos were so assiduously examined that it was difficult for the Reviewer to distinguish between normal and thallium-induced effects as reported; i.e., the multiple endpoints monitored and findings in both control and treated animals made

interpretation of results difficult. The investigators apparently were able to distinguish between barbering/cyclic hair growth and true alopecia.

Dr. Koller Comments

Some of the data from this study was/is difficult to interpret. Alopecia is a lesion known to occur in animals and humans exposed to toxic doses of thallium. Alopecia was present in all groups of animals in this study, including the controls. Clinical observations considered the majority of alopecia to be due to barbering, a normal behavior in rats. However, alopecia was noted in a percentage of each treatment group that was not considered to be due to barbering, more so in females than males. Although the lesions were "graded mild", they appeared to be more extensive in the females. The question to answer; is it an adverse health effect (biomarker of effect) or is it a biomarker of exposure? The degree of severity, non-consistent site-location of the alopecia (although as severity increases, more sites could become affected), and absence of skin (hair follicle) lesions (two females are discussed in number 6 below) are suggestive that alopecia at these doses is not an adverse health effect but rather a biomarker of exposure.

Two other effects open to interpretation were the lacrimation and exophthalmus observed in a dose-related fashion in the 90-day study. The effect was "graded present to mild", even in the high dose group. Ophthalmic examination of the eyes did not reveal any consistent lesions that were considered to be chemical related and no lesions were observed in the two highest treatment groups. Thus, although the effects did increase in a dose-related pattern, these effects were of an insufficient degree to be considered as an adverse health effect at the highest dose (0.25 mg/kg) tested in the study. Exophthalmus is an unusual lesion and it would be interesting to know the cause (edema?) of this lesion.

There were changes noted in some of the clinical chemistry profiles of the study. Although some of these changes were significant compared to the internal control animals, they were still mostly within historic reference ranges for their respective values. This coupled with the fact that histopathology did not reveal cellular lesions in tissue and/or organs that may have released the enzymes suggests that the change noted in serum chemistries does not represent an adverse health effect. The authors suggested that an increase in AST and LDH could represent heart lesions. However, these two serum enzymes (AST and LDH) are rather non-specific in nature and are indicative of muscle damage in general and are not specific for cardiac muscle. A serum enzyme often used for detection of damage to muscle (elevated after heart attacks) is creatine kinase that was not measured in this study. It is agreed by this reviewer that the other incidental lesions identified in this investigation were not related to the thallium sulfate exposure. Overall, the "Summary" of this study represents the data and is adequately interpreted.

4. Were the conclusions adequate? Are there any observations that were excluded from the conclusion that should have been included? More specifically, were there any observations that were excluded from the conclusions that are contradictory?

Dr. Davidson Comments

The reviewer differs with the investigators regarding the general interpretation of the results. The investigators did not consider exophthalmos, alopecia, and lacrimation observed in the 90-day study as toxicologically significant. These effects observed intermittently throughout the study showed a clear dose-response relationship in one or both sexes. Exophthalmos and lacrimation were generally graded minimal (present) to slight in severity and alopecia ranged up to severe grade. In addition, the incidence of miosis increased with dose particularly in males. Lacrimation, miosis, and exophthalmos may be signs of an effect of thallium administration on the nervous system. It is this reviewer's opinion that the effects on the eyes are treatment related and should be considered adverse.

Alopecia was observed during clinical observation in 12 females in the high-dose group; of these 12, 8 had evidence of alopecia at study termination, which was recorded for only 4 during necropsy. Two high-dose females showed atrophy of the hair follicle. Self-barbering was recorded during clinical observation in one of the females (#163) that showed atrophy of the hair follicle and alopecia was not recorded at necropsy in the other female (#234) that showed atrophy of the hair follicle. The investigators attributed alopecia to self-barbering or normal hair cycle, although the incidence of alopecia showed a clear dose-related trend. The high incidence of alopecia in the treated groups compared with the controls and the clear dose-related trend suggest that the alopecia was associated with administration of thallium. It is the opinion of this reviewer that alopecia is a treatment-related and an adverse effect. It appears that the collection of skin samples did not maximize the detection of atrophy of hair follicles. Skin likely to exhibit microscopic effects were not collected during necropsy of about half the high-dose females that showed alopecia during clinical observation at study termination.

The other effects on the skin such as rough coat, piloerection, and shedding showed strong dose-response relationships and should not be discounted. These observations may be signs of discomfort or stress, which is supported by observations of self biting and vocalization or they may be signs of an effect on the nervous system. It is the opinion of this reviewer that signs of rough coat, piloerection, and shedding taken together should be considered treatment related and adverse.

Dr. Talmage Comments

The investigators noted that alopecia, lacrimation, and exophthalmos increased in an apparent dose-related fashion, but did not consider them toxicologically significant. The Reviewer agrees that the effects may be minor, but these effects cannot be considered toxicologically insignificant. The Reviewer notes that diarrhea, a likely sign of thallium-induced gastrointestinal disturbance, was more prevalent in treated rats, especially males (Table 2 of study report).

Dr. Koller Comments

The Conclusions are adequately presented. The changes noted in clinical signs and blood chemistries are included. Alopecia, a lesion observed in thallium toxicity was concluded to be unrelated to thallium sulfate administration. This may be an inaccurate statement since there was a dose-response increase observed for this lesion. Nevertheless, at the doses tested for 90-days, it is not considered to be an adverse health effect. This reviewer agrees with the overall conclusion of the authors that "although some of the clinical signs as well as the alterations in clinical pathology parameters appear to be dose related, these effects were limited in magnitude and did not affect the health status of the treated animals". I disagree with the statement that the 0.25 mg/kg/day administered over a 90-day period represents a no observable effect level (NOEL). Effects were observed in this study, thus a NOEL was exceeded. I do agree, however, that the 0.25 mg/kg/day dose was a NOAEL; i.e., no observable adverse health effect level.

5. In your opinion, was this investigation properly planned, conducted, and reported? Are there any procedures, observation or analysis that would have added to the quality of this investigation?

Dr. Davidson Comments

The general procedure for this study appears to have followed standard protocol at the time it was conducted. As noted above thyroid hormone analysis would have indicated whether exophthalmos involved a thyroid hormone imbalance. However, exophthalmos, lacrimation, vocalization, and piloerection are also indicators of nervous system effect.

Dr. Talmage Comments

The investigation was properly planned, conducted, and reported. Short of repeating the study with a similar protocol in virus-free rats, but with microscopic examination of alopecia skin areas in all dose groups and perhaps ultramicroscopic examination of some tissues, the conduct was appropriate.

Dr. Koller Comments

As mentioned previously, this investigation followed standard protocol used in the mid-1980s to determine the subchronic toxicity of a chemical. It was properly designed, executed, and reported. For the time period this experiment was performed, the procedures, observations, and analysis were of sufficient quality to satisfy the objectives.

The preliminary study suggested the kidney was the systemic target organ for thallium toxicity. One would expect a noticeable increase in serum creatinine and blood urea nitrogen should the kidney be affected. The changes observed were slight (subtle) with no evidence of histopathological lesions. Using a higher dose in the experimental design would have perhaps shown an increase in alopecia, a dose-response for renal effects, or identified other target organ(s) of toxicity? There was also no evidence of myocardial effects although the authors used that as a rationale for the lung effects. Future studies should be designed to identify target

organ(s) of toxicity as well as the mechanism of toxicity to better understand the pathological pathway of thallium toxicity.

6. At the high dose, two female rats exhibited moderate to severe alopecia that could not attributed to self-barbering or normal cyclic hair growth. Histologic examination of skin samples from these high dose females showed atrophy of hair follicles. Hair loss (alopecia) is a well known effect of thallium poisoning in animals and humans. In light of this toxicological property of thallium, should the alopecia in high-dose females be considered toxicologically significant? Is the study authors' conclusion that the high-dose represents a NOAEL justified and supported by the study data?

Dr. Davidson Comments

Examination of the individual data indicates that alopecia noted in four high-dose females during clinical observation at study termination was not recorded during gross examination. Therefore, the incidence of atrophy of the hair follicles may have been higher if additional skin samples had been collected from animals showing alopecia at study termination. Therefore, this reviewer considers atrophy of hair follicles observed in two high-dose females toxicologically significant and should be considered when establishing the LOAEL/NOAEL for this 90-day thallium study. It also is the opinion of this reviewer that the NOAEL reported by the investigators is not justified by the data. Exophthalmos, lacrimation, and miosis also should be considered toxicologically significant and the study re-evaluated to consider these data in establishing the LOAEL/NOAEL for this 90-day thallium study.

Dr. Talmage Comments

The Reviewer considers the alopecia with atrophy of hair follicles in two female rats of the high-dose group (0.25 mg/kg/day) an effect of thallium treatment. The increased incidences of several signs - alopecia, lacrimation, exophthalmos, diarrhea - in all dose groups compared with the control groups suggests that a NOEL was not attained in this study. However, since most of the observed effects are reversible, the Reviewer suggests that the highest dose with the endpoint of atrophy of hair follicles is a border-line LOAEL.

Dr. Koller Comments

From my nearly 30 years of conducting basic biomedical research in rodents as a pathologist/toxicologist and over 15 years of experience in risk assessment, I would consider alopecia in the two female rats that was accompanied by atrophy of the hair follicles to be at the border of a LOAEL and a NOAEL. If I were using this study in risk assessment I would select the 0.25 mg/kg/day dose as the point of departure but would use it as a NOAEL rather than a LOAEL. The reason is that alopecia appears to be the most sensitive target organ for subchronic toxicity to thallium, it is an "early" (low grade) lesion, it is not a lesion that could be considered as an adverse health effect at that stage, and it certainly is not life threatening. One would expect the lesion to also be reversible.