

Interim Specific Ground Water Criterion
for Tri-ortho-cresyl phosphate (TOCP)

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Executive Summary

TOCP (tri-ortho-cresyl phosphate) is known to cause a specific neurodegenerative symptom known as organic phosphate-induced delayed neuropathy (OPIDN) in humans exposed by ingestion. TOCP has been the subject of much scientific research that has focused largely on the mechanism of induction of OPIDN. These studies have been complicated by the fact that rodents, the standard species used in toxicological research, have little or no susceptibility to TOCP-induced OPIDN. Chickens have been shown to be a better model for TOCP-induced OPIDN, although this creates uncertainties associated with extrapolation from avian species to humans.

There are no studies of chronic TOCP exposure in chickens. TOCP-induced OPIDN occurred in a sub-chronic (90-day) study in chickens (Prentice and Majeed, 1983), and provided the lowest NOAEL and LOAEL from among studies in chickens or rodents. The NOAEL from this study is consistent with that derived from another study (Roberts et al., 1983) that exposed chickens for the same length of time to a similar range of doses. TOCP produces male reproductive effects in rats as well as chickens, but the NOAEL for this effect in both species is larger than the NOAEL for OPIDN in chickens. The Reference Dose of 4×10^{-4} mg/kg/day is derived from Prentice and Majeed (1983). Application of the standard approaches and assumptions for derivation of the Interim Specific Groundwater Criterion yields a value of 0.003 mg/L or 3 µg/L.

Introduction – Scope of this Assessment

The purpose of this assessment is to derive an oral Reference Dose and an interim specific ground water criterion for tri-ortho-cresyl phosphate (TOCP). Although a soil remediation standard is not derived in this document, this Reference Dose can also serve as the basis for a soil remediation standard for the ingestion route of exposure. TOCP became well known as the result of mass poisonings in the 1930's when it was consumed as an adulterant in a bootleg alcohol extract of ginger. It was notable at the time for producing a progressive peripheral neuropathy in humans with a delayed onset known as "ginger jake." TOCP thus became the best known of a group of chemicals known to produce organophosphate-induced delayed neuropathy (commonly referred to as OPIDN). Given its history, the unusual nature of OPIDN, and the attempts over decades to understand the toxicological basis of OPIDN, a large medical and scientific literature dealing with TOCP has developed. However, much of this literature is concerned with the qualitative aspects of TOCP toxicology, with a focus on understanding the mechanism of OPIDN. To that end, most of the studies used high doses of TOCP known to reliably cause OPIDN with a single exposure or with exposure over an acute exposure duration, while relatively few studies were intended to quantify the dose-response relationship of TOCP toxicity. Therefore, this assessment will not attempt to review and summarize the full body of literature on TOCP toxicology. Rather this review focuses on those studies that are most relevant to a risk assessment based on chronic exposure, which are those that provide quantitative data on adverse health outcomes in the range of

doses that produce toxicity with subchronic to chronic exposure, but not with acute exposures.

An additional important aspect of the scope of this assessment is the unusual nature of the animal species utilized in some of the key studies. Toxicological studies commonly focus on rodents due to the ease with their ease of dosing, handling, housing etc., and due to the general close similarities of mammalian anatomy, physiology, and toxicology that allow reasonable extrapolation between rodents and humans. However, in the case of TOCP, rodents, in contrast to humans, are either not sensitive to TOCP-OPIDN or develop OPIDN only with very high doses. Thus, rodents have generally been recognized as not providing an acceptable model of TOCP induced OPIDN for humans (although as discussed below, there are other TOCP-mediated endpoints for which rodents may be a good model). Chickens (specifically hens), however, are sensitive to TOCP-mediated OPIDN at relatively low doses and have been identified as likely providing a good model for human dose-response for this effect (Craig and Barth, 1999; Baron, 1981; Lapadula et al., 1985). While unusual in the context of risk assessment, the use of this non-mammalian species appears to be justified. Therefore, the data for dose-response relationship for the OPIDN endpoint will be derived from studies in hens. Nonetheless, it should be noted that, as is also the case for most endpoints in which rodents are used as the model for human toxicity, there has been little attempt to investigate the quantitative relationship between effective doses of TOCP in chickens and those in humans. Thus, as is the case with interspecies extrapolation from rodents to humans, the underlying uncertainties in interspecies extrapolation from chickens to humans will be addressed in the context of the interspecies uncertainty factor adjustment.

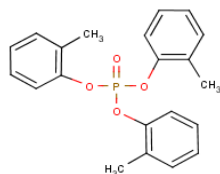
Physical and Chemical Properties

(Data accessed at:

<http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~gq3RdE:1>, accessed 8/31/10,

and

<http://chem.sis.nlm.nih.gov/chemidplus/jsp/common/PhysicalProperties.jsp?calledFrom=>



[lite](#), accessed 8/31/10).

CAS Number - 78-30-8

Synonyms - Tri-ortho-cresyl phosphate; Tris(o-cresyl)-phosphate

MW - 368.37

Melting point – 11 °C

Boiling point – 410 °C

Water solubility – 0.102 mg/L

Soluble in alcohol, benzene, ether

Log K_{ow} - 5.11

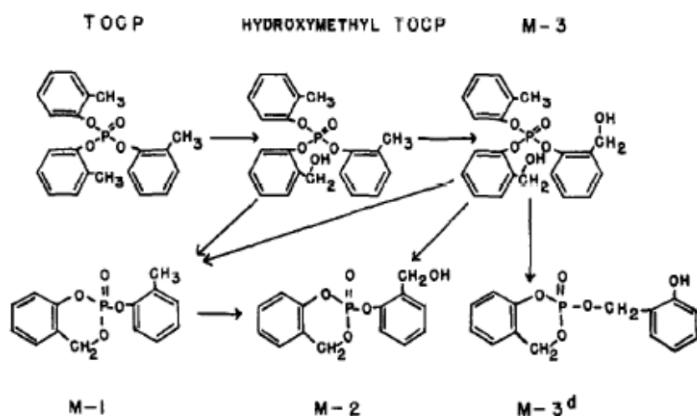
Density - 1.1955 @ 20 DEG C

Production, Use, Occurrence

Historically, TOCP occurred in the production of mixed tri-cresyl phosphates (i.e., o-, m- and p-cresyl phosphate) by the reaction of phosphate oxychloride with a mixture of cresols. Tri-cresyl phosphate has been used as a plasticizer for chlorinated rubber, vinyl plastics, polystyrene, polyacrylic and polymethacrylic esters; as an adjuvant in milling pigment pastes; as a solvent and a binder in various natural resins; as a lubricant in synthetic lubricants and gasoline; as a hydraulic fluid; and as a fire retardant (NIOSH, 1977). It does not appear that TOCP was manufactured or used extensively for industrial purposes as a pure substance. However, during Prohibition, TOCP, as a more-or-less pure compound, was used as an additive in an attempt to circumvent U.S. Treasury Department restrictions on the sale of a high alcohol-content patent medicine, variously called Jamaica Ginger, Jake, or Ginger Jake. In 1930, consumption of the adulterated substance resulted in 30,000-50,000 cases of severe peripheral neuropathy in the U.S., commonly called ginger jake (http://en.wikipedia.org/wiki/Jamaica_ginger accessed 10/18/10).

Pharmacokinetics and Metabolism

TOCP is initially metabolized to saligenin cyclic-o-tolyl phosphate (SCoTP) (compound M1 in the following figure) through the loss of one of the ortho-cresols followed by cyclization of the phosphate with the methyl group of one of the remaining cresols. This compound is reactive and can bind to the active site on various neurologically active esterases including acetylcholinesterase and NTE (neuropathy target esterase), which appears to play a role in OPIDN.



Intestinal fluid containing metabolized TOCP extracted following an oral dose was reported to be 20,000 times more potent (for induction of OPIDN?) than the parent compound (Eto et al., 1962). Clinical and histopathological manifestations of OPIDN are observed with subcutaneous injection of SCoTP (Casida et al., 1961). This suggests that SCoTP (or possibly one of its subsequent metabolites) is the ultimate toxicant.

In hens given a single, non-neurotoxic oral dose of ^{14}C -TOCP and followed for 5 days (**Abou-Donia et al., 1990**), the maximum accumulation in most organs occurred between 0.5-1 days after dosing. The maximum accumulation was found in the ventricular (gizzard) lining, bile, large intestine, gall bladder, and crop (i.e., in the organs of the gastrointestinal tract). Levels in neurologic tissue (brain, spinal cord, sciatic nerve) as well as erythrocytes were 1-2 orders of magnitude lower than in the gastrointestinal tract organs. The ^{14}C in adipose tissue accounted for only about 0.1% of the total recovered ^{14}C after 0.5 days. However, after a decline at day one, the label in adipose tissue continued to increase. By day-5, it accounted for about 14% of the remaining ^{14}C in the body. The relative retention of the TOCP and/or its metabolites in adipose tissue may contribute to the delayed nature of the neurotoxicity. Elimination in combined urine-feces (excreted together in chickens) occurred with a half-life of approximately 1.5 days. The authors note that since this dose was not neurotoxic, it is possible that the pharmacokinetics of a larger, neurotoxic dose could differ.

In a study with a similar experimental design (Suwita and Abou-Donia, 1990), both the whole-body, and the plasma half-life of TOCP label in chickens were 53 hours. In comparison, the whole body and plasma half-lives for rats were lower (63% lower for plasma), and the concentration of SCoTP was about an order of magnitude lower than in chickens. These pharmacokinetic differences may partly explain the different susceptibilities of chickens and rats to OPIDN.

Acute Toxicity

The human adult lethal dose has been reported as 1-10 g ((**Clayton and Clayton, 2000**)). The minimum paralytic dose (i.e., the minimum single dose capable of producing OPIDN) in humans is estimated at 10-30 mg/kg ((**Clayton and Clayton, 2000**)). Doses capable of producing OPIDN produces either no immediate symptoms or GI disturbances. After several hours, cramps in the legs may develop along with some numbness in hands and feet. Bilateral foot drop develops after several additional hours. After several days, weakness in hands and wrists develops. Muscle weakness progresses over a period of weeks to months. The extent of paralysis depends on the amount ingested and the dose rate (**Clayton and Clayton, 2000**). In general, while some acute symptoms reflect cholinesterase inhibition, effects characteristic of OPIDN (e.g., ataxia, paralysis, demyelination) do not appear to be related to cholinesterase inhibition and can occur in the absence of cholinesterase inhibition.

In hens, a single oral dose of 1 ml/kg (1.2 g/kg) produced signs of OPIDN by eight days post-dosing and severe paralysis within two weeks (Cavanagh, 1954).

A single oral dose of 50 mg/kg does not result in OPIDN or cholinergic effects in hens (**Abou-Donia et al., 1990; Suwita and Abou-Donia, 1990**). Given the estimated minimum single human dose capable of producing OPIDN (above), these observations suggest that chickens may be less sensitive to acute exposure to TOCP than humans.

Ehrich et al. (2001) state as a generalization that OPIDN in chickens affects adult chickens only, while younger chickens (55-60 day) old are not susceptible. However, this observation is based on a summary of studies that attempted to induce OPIDN using

organophosphates other than TOCP that are effective in adult chickens. Therefore, it is not clear if this generalization applies equally to TOCP.

In male and female CD-1 mice (25-30 g body-weight), a single oral dose of TOCP ranging from 580 to 3,480 mg/kg followed by observation for up to 14 days post-dosing, did not produce clinical symptoms of either cholinergic poisoning or OPIDN ataxia despite the very high doses compared to the effective acute doses in chickens (**Veronesi et al., 1991**). Nonetheless, dose-dependent spinal neuropathology was observed upon pathological and histopathological examination in at least some sections of the spinal cord at all doses. However, the dose dependence was observed against a high inter-individual variability. Although NTE inhibition occurred with 50-70% inhibition at 44 hr, there was no clear dose-response relationship for this effect and NTE inhibition did not correlate with spinal cord pathology. In addition, in some animals, myelin structure changes and swollen axons were observed in peripheral nerves at doses greater than 1,160 mg/kg. There was evidence of axon regeneration at 580 mg/kg. The authors suggest that axonal regeneration may be responsible, at least partly, for rodent resistance to OPIDN.

SPF-Wistar rats were exposed to a single oral dose of 1,500 mg/kg TOCP at 12 weeks of age (**Inui et al., 1991**). The authors identify this as the LD₅₀. Atropine was co-administered pre- and post-TOCP to block the acute cholinergic response. No OPIDN-related responses were observed (including ataxia or paralysis), although cholinergic effects ((sedation, tremor, salivation, dacryorrhea (excessive tearing), urine soiling) were observed despite the atropine administration. The significance of this observation is not clear. Consistent with the observation in mice by Veronesi et al. (1991), after 2 weeks (in surviving animals) spinal cord nerve fiber degeneration – axonal swelling, myelin debris, macrophage infiltration into myelin sheaths, and axonal vacuoles (indicative of fiber disruption) were observed despite the lack of clinical OPIDN symptoms.

Chronic/Subchronic Toxicity

OPIDN and Other Neurotoxic Effects

Chickens

Somkuti et al. (1988) dosed three 24-week old chickens (sex not specified) with body weight 2.0-2.4 kg with 100 mg/kg/day TOCP once per day for 18 days. The chickens were observed daily for signs of acute neurotoxicity (salivation, diarrhea, ocular discharge, tremor) and delayed neurotoxicity (gait disturbance, hindlimb splay, paralysis). Ataxia was observed on day 7 and paralysis (consistent with OPIDN) on day 15. Histopathological changes included spinal axon degeneration, axonal swelling and demyelination. Brain acetylcholinesterase activity was decreased by 68% and brain NTE activity was decreased by 64%.

As part of a study primarily focused on leptophos (an OPIDN-inducing agent), **Abou-Donia and Graham (1979)** administered TOCP to hens as a positive control. Five 19-month old hens with a mean body weight of 1.36 kg were given a daily dose of 10 mg/kg of TOCP orally for 4 months followed by 4 months of observation. Ataxia was observed

in 1 of 5 hens at day 10 and in 4 of 5 at days 31-39. The hen that showed signs of ataxia at day 10 developed severe ataxia at day 20 and died at day 23. In the remaining 4 hens, the ataxia became severe during the post-dosing period, and in two of these hens, the ataxia progressed to near paralysis. In all cases, histopathological examination showed clear spinal axon degeneration with demyelination. Based on this study, 10 mg/kg/day is a clear effect dose with subchronic exposure.

In a study with somewhat similar design, **Abou-Donia et al. (1979)** administered a daily oral dose of 10 mg/kg/day of TOCP for 3 months to five 19-month old hens with an approximately 2 kg body weight followed by one month of observation of the surviving hens. As above, this study was focused primarily on DEF (a putative OPIDN-inducing agent), and TOCP was given as a positive control for OPIDN. Four hens developed mild ataxia at days 27-34 that progressed to gross ataxia. One hen developed paralysis and died on day 5 without ataxia. Two animals developed paralysis on days 54 and 77 and died within 2 days. Three of the animals had clear spinal cord degeneration and one without spinal cord pathology had peripheral axon pathology and myelin degeneration. This study is consistent with the previous study in showing clear OPIDN with subchronic exposure at 10 mg/kg/day.

Roberts et al. (1983) reported on a study carried out in accordance with the USEPA 90 day neurotoxicity study design. Hens approximately 12 months old and with a body weight greater than 2 kg were dosed orally for 90 days (the method of dosing is not provided) with TOCP at the following doses (the number of animals at each dose is given in parenthesis):

Doses (mg/kg/day):

Study 1 - 0 (n=12), 1.25 (12), 2.5 (12), 5.0 (12), 10.0 (12), 20 (12)

Study 2 - 1.0 (20), 5.0 (20), 10.0 (20)

Study 3 - 7.5 (10)

Food consumption and body weight were recorded over 7 day periods and body weight was measured at least once per week. Assessment of ataxia was based on observation of individual birds relative to an 8-grade scale. No statistical analysis was provided.

The authors report the result as the combined studies data. No neurotoxicity was observed for doses ≤ 2.5 mg/kg/day (i.e., all animals had 0-grade ataxia). At 5.0 mg/kg/d, approximately 75% had grade 1 ataxia (doubtful-slight incoordination, approximately 2% had grade 2 ataxia (slight incoordination, occasional stumbling or wing drooping), 20% had grade 3 ataxia (frequent incoordination or stumbling). The severity of ataxia increased consistently with increasing doses.

In this study, 5.0 mg/kg/day is a clear effect level and 2.5 mg/kg/day is a NOAEL.

Prentice and Majeed (1983) dosed hens with a body weight of approximately 2 kg, of unspecified age (but, adults, based on body weight) with TOCP in corn oil daily for 90 days at the following doses (the number of animals per dose group is given in parenthesis):

0 mg/kg/day (n = 35), 1.0 (10), 1.25 (5), 2.5 (5), 5 (17), 7.5 (9), 10 (19), 20 (8).

Ataxia was assessed on a graded 8-step scale. Histopathology was carried out on spinal cord and peripheral nerve fibers. Nerve damage was evaluated on a scale of 1-4 for each of four spinal regions, and the scores were added to produce a single measure of spinal nerve damage for each animal (i.e., a maximum possible score of 16).

Ataxia was reported in hens with doses ≥ 5 mg/kg/day. No ataxia was seen in the hens receiving the 1.0, 1.25 or 2.5 mg/kg/day doses.

Mean values for spinal histopathology scores for the controls, and 1.0 and 1.25 mg/kg/day dosed hens were 1.7, 0.7 and 1.8. None of the individual scores at these doses exceeded the maximum score (~4.8) among the control hens. Mean histopathology scores increased with doses greater than 1.25, with mean values for those doses of 2.5 mg/kg/day, 3.2; 5.0 mg/kg/day, 4.8; 7.5 mg/kg/day, 8.4; 10.0 mg/kg/day, 9.6; 20.0 mg/kg/day, 12.4. The mean scores for doses ≥ 2.5 mg/kg/day were significantly different from controls ($p < 0.01$). Peripheral nerve histopathology damage was evident for doses ≥ 7.5 mg/kg/day but with a lower intensity than for spinal cord. The ataxia observed in this study was qualitatively similar to that seen with acute exposures.

The NOAEL, for ataxia in this study is 2.5 mg/kg/day and the NOAEL for spinal nerve damage is 1.25 mg/kg/day.

The lowest NOAEL from the studies of chickens is 1.25 mg/kg/day, and the lowest LOAEL is 2.5 mg/kg/day (Prentice and Majeed, 1983). Note, however, that there are no reports of studies of chronic TOCP exposure in chickens.

Rodents

Ehrich et al. (2004) dosed Long-Evans rats (12 per dose group) with 7 doses of 75, 150 and 300 mg/kg over a 14 day period. These doses correspond to nominal doses of 37.5, 75 and 150 mg/kg/day.

There was a significant decrease in body weight at 37.5 mg/kg/day and at 150 mg/kg/day, but not at 75 mg/kg/day. A significant decrease in forelimb grip strength was reported at 150 mg/kg/day. Grip strength data were not reported at lower doses.

Blood acetyl cholinesterase (AChE) activity was decreased by 46, 45 and 64%, respectively, at the three doses. However, statistical significance was not specified. There was also a decrease in brain AChE in various brain regions at all doses (statistical significance not specified). There was also significant inhibition of brain NTE in various brain regions at all doses. Histopathological abnormalities were confined to swollen myelinated spinal axons in 1 of 5 animals at 150 mg/kg/d.

The LOAEL for this study is 37.5 mg/kg/day with no NOAEL. The absence of clinical neurological effects including ataxia should be noted.

Somkuti et al. (1987a) conducted a 14-day range-finding and 63-day study of TOCP in 10-11 week-old Fischer 344 male rats. The primary focus of this study was to examine

effects on testes and sperm. Those results are reported below under Reproductive and Developmental Toxicity. Neurologic effects were also monitored and those are reported here.

In the 14-day study, rats (8/dose group) were dosed by gavage at 0, 100, 200, 400, 800, or 1,600 mg/kg/day TOCP in corn oil. There was nearly 100% mortality at doses \geq 800 mg/kg/day, 70% mortality at 400 mg/kg/day, and 60% mortality at 200 mg/kg/day within 2-5 days. However, there was no mortality at 100 mg/kg/day. The specific cause of mortality was not reported. Cholinergic toxicity was observed in all animals at doses $>$ 200 mg/kg/day, but no signs of cholinergic toxicity were observed at 100 mg/kg/day.

In the 63-day study, 10 animals per dose group were dosed with 0, 10, 25, 50, 75, 100 mg TOCP/kg/day. Body weight was monitored and daily observation was carried out for neurologic dysfunction and signs of acute cholinergic toxicity (e.g., salivation, tremors, diarrhea, ocular discharge). No mortality occurred. There was decreased weight gain at \geq 50 mg/kg/day despite little change in food intake (statistical significance was not reported). No neurologic signs were reported.

The NOAEL for neurological effects in this study appears to be 25 mg/kg/day based on decreased weight gain. However, in the absence of statistical data, that cannot be confirmed.

The main focus of the **Lapadula et al. (1985)** study was to compare the toxicity of acute and chronic doses of TOCP. Harlan Sprague-Dawley male mice (5 per dose group) were dosed with either two single 1,000 mg/kg doses, 21 days apart, or with daily doses of 225 mg/kg/day for 270 days. The animals from both dosing regimens and control mice were observed for a total of 270 days.

The control mice and the 2 x 1,000 mg/kg mice had similar body weight increased from their pre-treatment weights. In the chronically exposed (225 mg/kg/day) mice, body weight averaged 29% less than the control mice. No delayed neurotoxicity was observed in the 2 x 1,000 mg/kg mice. The first signs of delayed neurotoxicity appeared in the chronically exposed mice in the 8th week of dosing and progressed to severe ataxia in 3 of 5 mice by day 230 and to hind limb paralysis in all chronically exposed mice by day 270.

Spinal cord (axon and myelin) degeneration was seen in the 225 mg/kg/day mice, but not in the 2 x 1,000 mg/kg mice. In addition, there was clear peripheral nerve degeneration in 2 of 5, 225 mg/kg/day mice and equivocal changes in the remaining three.

There was significant inhibition of brain AChE and NTE in the 225 mg/kg/day mice, but not in the 2 x 1,000 mg/kg mice.

It is clear that at these doses, mice are more sensitive to long-term exposure than to a much larger acute exposure. However, comparison of these doses does not provide a basis for predicting the effects of chronic exposure at even lower doses.

No other chronic duration studies of TOCP in either rodents or chickens were located.

Reproductive and Developmental Toxicity

Somkuti et al. (1987a) conducted a 14-day range-finding study and 63-day study of TOCP dosing in male Fischer-344 rats. This study design and doses are presented above with respect to neurotoxic endpoints. For reproductive tract/testicular/sperm effects, the 63-day study corresponded to the 63-day seminiferous epithelium cycle (49 days) plus the epididymal transit time (14 days). In the 14-day study, with 8 animals exposed at each dose, two animals per dose were evaluated for testicular histopathology and the remaining 6 per dose evaluated for sperm density. In the 63-day study, with 10 animals exposed at each dose, 5 animals were used for testicular histopathology and 5 animals were used to conduct various enzyme and hormone studies.

In the 14-day study, there was a dose-dependent decrease in cauda epididymal sperm density with a 50% decrease at the lowest dose (100 mg/kg/day). Abnormal testicular histopathology was observed at all doses.

In the 63-day study, there was a statistically significant decrease in relative testes weight at the two highest doses (75 and 100 mg/kg/day). Sperm motility was significantly decreased at ≥ 50 mg/kg/day with no mobile sperm observed. At 25 mg/kg/day, there was an approximate 50% decrease in sperm motility although this decrease did not reach the level of statistical significance. Cauda epididymal sperm density was significantly decreased at ≥ 50 mg/kg/day with a decrease of approximately 57% at 50 mg/kg/day. At 25 mg/kg/day there was a non-statistically significant decrease of approximately 30%. In microscopic testicular cross-sections of epididymal tubules, the structure in animals dosed at 10 mg/kg/day was indistinguishable from that of control animals. At 25 mg/kg/day, 3 of 5 animals had a disorganized germ cell array and inclusion droplets. The disorganization was consistently overt at ≥ 50 mg/kg/day and immature germ cells and multinucleated giant cells were noted in the tubules.

Non-specific (testicular) esterase activity was significantly decreased at ≥ 10 mg/kg/day, and NTE activity was significantly decreased at ≥ 50 mg/kg/day. There was no dose-related decrease in testosterone.

The authors identify the LOAEL from this study as 25 mg/kg/day based on histology (germ cell array organization and changes in cellular morphology). The NOAEL is 10 mg/kg/day.

Somkuti et al. (1987b; 1991) report results consistent with those summarized above (Somkuti et al., 1987a) extending to complete absence of germinal cells when male Fischer-344 rats were exposed at the single dose level of 150 mg/kg/day for up to 21 days.

Somkuti et al (1987c) also investigated the male reproductive toxicity of TOCP in roosters. Three-years-old adult roosters (n = 10) were dosed daily for 18 days with 100 mg/kg/day TOCP in corn oil. These were compared to 10 untreated control roosters.

Sperm motility was nearly completely absent in the TOCP-dosed roosters, and there was a significant disorganization of the seminiferous epithelium in 5 of 10 treated roosters.

No consistent pattern of effects was seen in the remaining 5 treated roosters. Testis NSE (non-specific esterase) activity was inhibited by 17% compared to controls, and testis NTE activity was decreased by 85%. The function of these enzymes in the testes is not clear.

Tocco et al. (1987) dosed pregnant Long-Evans rats with TOCP in corn oil at 0, 87.5, 175, 350 mg/kg/day (n = 10-18/dose) for 13 days starting with GD 6. They were sacrificed at day 21. Fetuses in-situ, and resorption sites were counted. Gross abnormalities were documented and pathology and histopathology was performed.

There was 28% (5/18) maternal mortality at 350 mg/kg/day with no mortality occurring at other doses. Death was associated with toxicity from acute cholinesterase inhibition. No maternal mortality or overt maternal toxicity was observed at 0, 87.5 or 175 mg/kg/day. There was no significant difference in body weight among dose groups, including comparison to the controls.

There was no significant difference in the number of implants/litter, percent pre-implantation loss, or percent resorption. However, while not statistically significant, there was a three-fold increase in resorption at 350 mg/kg/day compared to controls. There was no significant difference in fetal weight in dosed animals, but all dose groups had higher mean fetal weight than the controls.

There were no significant dose-related skeletal malformations. Although there was a ~2% incidence in malformations at 350 mg/kg/day, there was a similar incidence in the controls. There was no significant difference across doses or in comparison to controls skeletal variations.

With the possible exception of a non-significant increase in fetal resorption at 350 mg/kg/day, there does not appear to be any fetal reproductive/teratogenic effects within the tested dose range.

Immunotoxicity

Ehrich et al. (2004) evaluated several immunologic parameters in male Long-Evans rats dosed with 7 doses of 75, 150, or 300 mg/kg TOCP over 14 days. Calculated on a nominal mg/kg/day basis, these doses correspond to 37.5, 75, and 150 mg/kg/day. There were no significant differences from controls in thymus weight, thymus cell count, spleen weight, spleen cell count, or thymus or spleen cell surface markers.

Brinkerhoff et al. (1981) dosed male Swiss-Webster mice with a mean body weight of 28 g (age not specified) with 0, 5, 50, or 500 mg/kg TOCP by gavage once per week for 13 weeks.

Several different assays of immunoreactivity/competence were conducted. In the splenic lymphocyte culture assay, lymphocytes extracted from spleen after in-vivo exposure were cultured in the presence of two mitogens and bacterial lipopolysaccharide, and ³H thymidine uptake was monitored. In the splenic plaque formation assay, lymphocytes

were cultured as above and incubated with sheep erythrocytes, and plaques were counted. In the quantitative immunoelectrophoresis assay, IgA, G and M were measured.

No significant change was observed in spleen or thymus weight. The response to mitogens was inconsistent with respect to both dose and time. For one mitogen (PHA), there was a significant decrease seen only at week 13 and only at the two lower doses. For the other mitogen (PWM), there was a significant increase in response at week 4 at the 50 mg/kg dose, and significant decreases in response at 500 mg/kg at week 8, and at 5 and 500 mg/kg at week 13. There was no significant change in response to the bacterial lipopolysaccharide challenge. There was no significant change with dose in plaque formation or in the immunoglobulin content of serum.

From this study there does not appear to be a clear dose-response for TOCP with respect to immunologic effects. A central hypothesis of this study was that the neurotoxic effects of TOCP were secondary to immunotoxic effects. This does not appear to be the case.

Genotoxicity/Mutagenicity and Carcinogenicity

Mentzchel et al. (1993a) reported that TOCP is negative in the *S. typhimurium* TA100 mutagenic assay without metabolic activation by rat liver microsomes, but positive in the presence of liver microsomes. Mentzchel et al. (1993a) state that the metabolism of TOCP to the active SCoTP metabolite is mediated by the P450 system. This is consistent with the observed mutagenicity in this system in the presence of the liver microsomes.

Mentzchel et al. (1993b) reported that TOCP produced DNA adducts in *S. typhimurium* strain TA100 and cultured hepatoma cells. They also reported DNA adduct formation in the kidney, liver, heart, and lung of Fischer 344 rats dosed orally with 50 mg/kg/day for 10 days. Adducts were not found in the brain or testes.

These studies suggest that TOCP has mutagenic potential. However, there are no data on its carcinogenicity.

Development of the Toxicity Factor for TOCP

Table 1 presents a summary of the various toxic endpoints for the sub-chronic and chronic exposures presented above.

Table 1. Summary of Sub-Chronic, Chronic, Reproductive, and Immunological Studies of TOCP Toxicity

Species	Duration	Adverse Effect	NOAEL	LOAEL	Study
Sub-Chronic and Chronic Exposure Studies					
Chickens (hens)	18 d	ataxia; paralysis; axonal degeneration, swelling and	—	100 mg/kg/day (single dose study)	Somkuti et al. (1988)

Species	Duration	Adverse Effect	NOAEL	LOAEL	Study
		demyelination; decreased brain esterases			
Chickens (hens)	4 mos. (followed by 4 mos. of observation)	severe ataxia; spinal axon degeneration with demyelination	—	10 mg/kg/day (single dose study)	Abou-Donia and Graham (1979)
Chickens (hens)	3 mos. (followed by 1 mo. of observation)	ataxia; paralysis; spinal axon, and peripheral nerve degeneration with demyelination	—	10 mg/kg/day (single dose study)	Abou-Donia et al. (1979)
Chickens (hens)	90 d	at LOAEL - ataxia ranging from slight incoordination to pronounced ataxia with frequent stumbling	2.5 mg/kg/day	5.0 mg/kg/day	Roberts et al. (1983)
Chickens (hens)	90 d	ataxia; spinal axon degeneration, inconsistent peripheral nerve pathology	1.25 mg/kg/day	2.5 mg/kg/day (for spinal nerve pathology)	Prentice and Majeed (1983)
Rats (Long-Evans)	7 doses over 14 d	significantly decreased body weight	—	37.5 mg/kg/day (nominal daily dose including non-dosing days)	Ehrich et al. (2004)
Rats (male – Fischer-344)	14 d range finding 63 d	Decreased weight gain in 63 d study (but no statistical analysis); cholinergic toxicity in range finding study only	25 mg/kg/day (200 mg/kg/day in range finding study)	50 mg/kg/day (400 mg/kg/day in range finding study)	Somkuti et al. (1987a)
Mice (Harlan Sprage-Dawley – male)	270 d	ataxia; spinal paralysis; axon and myelin degeneration; peripheral nerve degeneration;	—	225 mg/kg/day (single dose study)	Lapadula et al. (1985)

Species	Duration	Adverse Effect	NOAEL	LOAEL	Study
		inhibition of brain esterases			
Reproductive Studies					
Rats (male – Fischer-344)	63 d	disruption in germ cell array organization; changes in epididymal tubule cellular morphology	10 mg/kg/day	25 mg/kg/day	Somkuti et al. (1987a)
Roosters	18 d	decreased sperm motility; disorganization of seminiferous epithelium	—	100 mg/kg/day (single dose study)	Somkuti et al (1987c)
Pregnant Long-Evans rats	gestational-day 6-18	Maternal lethality (cholinergic). No statistically significant fetal effects	175 mg/kg/day	350 mg/kg/day (maternal)	Tocco et al. (1987)
Immunological Studies					
Rats (Long-Evans - male)	7 doses over 14 d	no effect on immunological parameters	150 mg/kg/day (freestanding NOAEL calculated based on daily dose calculated including non-dosing days)	—	Ehrich et al. (2004)
Mice (Swiss-Webster – male)	13 wks – once per week	inconsistent changes in response to mitogen challenge – no clear adverse effects	cannot be determined	cannot be determined	Brinkerhoff et al. (1981)

Critical Study

The lowest NOAEL (1.25 mg/kg/day) and the lowest LOAEL (2.5 mg/kg/day) based on symptoms of OPIDN in hens, are derived from the subchronic (90 day) study of Prentice and Majeed (1983). As discussed previously, chickens and humans, but not most species and strains of rodents, develop OPIDN. In the studies of TOCP exposure in rodents,

either no OPIDN was observed, histopathological changes associated with OPIDN were observed but without ataxia or paralysis, or full symptoms of OPIDN were observed, but only with a long delay and at doses much higher than those producing OPIDN in chickens. Additionally, based on observations from acute data discussed above, it appears that chickens may be somewhat less sensitive to the OPIDN toxicity of TOCP than humans.

TOCP also produces male reproductive effects in both rats and roosters, these effects have only been observed at higher doses than the NOAEL and LOAEL for OPIDN observed in the 90-day Prentice and Majeed (1983) study in hens. On the basis of these considerations, the Prentice and Majeed (1983) study is identified as the critical study for the development of the Reference Dose for TOCP. It is noted that this study is limited by the use of a non-mammalian model (i.e., chickens) and by the small number of animals exposed at the NOAEL (5). The use of this NOAEL, however, is supported by the 90-day study of Roberts et al. (1983) that identified a NOAEL of 2.5 mg/kg/day in chickens. In that study, 20 animals were exposed at 1.0 mg/kg/day and 12 animals each were exposed at 1.25 and 2.5 mg/kg/day. While the LOAEL dose in the Prentice and Majeed (1983) is the same as the NOAEL in the Roberts et al. (1983) study for the same duration of exposure, this should not be seen as a serious discrepancy. These values vary only by a factor of two, and it is not known whether the strains of chickens and their handling in each study were identical.

Point of Departure (POD) for Derivation of a Reference Dose

Prentice and Majeed (1983) do not present their data in a manner that allows it to be used for benchmark dose modeling. Specifically, neither the raw neuro-histopathology data for individual animals, nor the standard deviations for those scores by dose are provided. Figure 1 in their publication presents the dose-group data graphically, but there is insufficient detail to reconstruct the quantitative dose-response data. Therefore, the NOAEL of 1.25 mg/kg/day from that study is identified as the POD for derivation of a Reference Dose.

Application of Uncertainty Factors

LOAEL - NOAEL - The critical study yields both a LOAEL and a NOAEL for the same endpoints. Therefore, no uncertainty factor is needed to estimate a NOAEL. It should be noted that the NOAEL is lower than the LOAEL by a factor of only two.

Sub-Chronic – Chronic - The only chronic duration study of oral TOCP administration (Lapadula et al. (1985) employed only a single dose that was more than two orders of magnitude higher than the NOAEL from Prentice and Majeed (1983). From comparison of the acute and sub-chronic studies presented here, it is clear that with repeated exposure to TOCP, sub-clinical toxicity can be cumulative. Thus, it is reasonable to assume that the 90-day NOAEL derived from Prentice and Majeed (1983) could be an effective dose with a longer duration (i.e., chronic) exposure. Therefore a full uncertainty factor of 10 is appropriate to estimate the chronic NOAEL.

Average Animal – Average Human - The extrapolation from chickens to humans warrants the full default uncertainty factor of 10.

Average Human - Sensitive Human - This is a standard adjustment that addresses individuals and groups within the human population with greater susceptibility. Although some data from chickens suggests that young animals are not susceptible to OPIDN, there are no data on factors affecting sensitivity to TOCP in humans and, in fact, no data on human sub-chronic or chronic toxicity from TOCP. In the absence of data on factors affecting sensitivity in humans, the full default factor of 10 is applied.

Database Insufficiency - There are two adequate studies addressing male reproductive toxicity, one of which is a mammalian (rat) study. There is also one study addressing mammalian (rat) gestational toxicity/teratogenicity in a single generation study in a single species and a single strain. In addition, there are few data to suggest how the dose response for OPIDN in chickens corresponds to that for humans. The lack of data specific to chicken-human extrapolation, the lack of developmental studies in additional species and/or strains of rodent, and the lack of a two-generation reproductive study warrant an overall database uncertainty adjustment of 3.

Cumulative Uncertainty Factor Adjustment - The cumulative uncertainty factor adjustment is calculated as:

$$UF_{\text{total}} = UF_{\text{sub-chronic - chronic}} \times UF_{\text{sensitive human}} \times UF_{\text{animal-human}} \times UF_{\text{database insuff.}} = 10 \times 10 \times 10 \times 3 = 3000$$

Calculation of the Reference Dose - The Reference Dose is calculated as:

$$POD/UF_{\text{total}} = 1.25 \text{ mg/kg/day}/3000 = 0.0004 \text{ mg/kg/day} = 4 \times 10^{-4} \text{ mg/kg/day.}$$

Confidence in the Derivation

The main limitation in the database supporting the derivation of a Reference Dose for TOCP is the lack of an appropriate mammalian model for the dose-response for the critical effect, OPIDN. The USEPA's IRIS database does not contain any Reference Doses based primarily on studies in chickens. However, the IRIS database also does not address any substances for which OPIDN is the critical effect. There is a general lack of toxicological data relating responses in chickens, particularly neurotoxic responses, to those in humans. This limits the confidence with which quantitative data from chickens can be extrapolated to humans. However, given the extensive historical data on TOCP poisoning in humans leading to OPIDN, it is possible to identify the response in chickens as qualitatively similar to that in humans. Based on acute exposure data, the quantitative dose-response in chickens appears to be somewhat less sensitive than in humans. Because there do not appear to be any human epidemiological or medical data relating to sub-chronic or chronic exposures, it is not possible to evaluate the relationship between the dose-response in chickens and humans with sub-chronic or chronic exposure. In addition, the number of animals in the critical study exposed at the NOAEL was small – i.e., 5. This limits the reliability of the NOAEL. That is, it is unlikely that a 10% response would have been seen at that dose. The inability to conduct benchmark dose modeling of these data further limits the reliability of using the NOAEL as the POD. As

discussed above, this concern is mitigated somewhat by the fact the Roberts et al. (1983) study that exposed chickens for the same length of time, found no adverse responses at a dose (1.25 mg/kg/day) below the NOAEL from the critical study despite exposing a larger number of animals (20).

For these reasons there is low-medium confidence in the Reference Dose.

Calculation of the Interim Specific Groundwater Criterion

The interim specific groundwater criterion (IGC) is calculated as:

$$\text{IGC} = ((\text{RfD} \times \text{BW} \times \text{RSC})/\text{I})$$

Where

IGC = Interim Specific Groundwater Criterion (mg/L)

RfD = Reference Dose (4×10^{-4} mg/kg/day)

BW = assumed body weight (70 kg)

RSC = Relative Source Contribution from drinking water (0.2 as a default value in the absence of specific data)

I = assumed daily water intake (2 L/day)

Given these inputs, the IGC is derived as:

$$\text{IGC} = ((4 \times 10^{-4} \text{ mg/kg/day} \times 70 \text{ kg} \times 0.2)/2 \text{ L/day}) = 2.8 \times 10^{-3} \text{ mg/L.}$$

Rounding to one significant figure gives an IGC of 0.003 mg/L or 3 µg/L.

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