Interim Specific Ground Water Criterion for Tricresyl Phosphate (TCP) Mixtures

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Background

The Office of Science submitted the final version of the Interim Specific Reference Dose and Ground Water Criterion for Tri-ortho-cresyl phosphate (TOCP) to MaryAnne Kuserk of the NJDEP Site Remediation Program (SRP) on November 16, 2010. That document recommended an RfD for TOCP of 4 x 10^{-4} mg/kg/day and Interim Specific Ground Water Criterion of 3 µg/L based on protection against organophosphate-induced delayed neuropathy (OPIDN).

Subsequent discussions with SRP indicated that Johnson & Johnson stated that TOCP was present on site in only one sample at 18.3 µg/L. All other detects of this family of compounds were for "tricresyl phosphates – total." Based on this information, the Office of Science was asked to develop an Interim Specific Ground Water Criterion for total tricresyl phosphates (TCP).

The submissions from Johnson & Johnson submitted by ARCADIS were reviewed by Dr. Alan Stern (Office of Science). These consist of two short documents: Attachments 1 and 2 of a July 11, 2005 report entitled, Supplemental Remedial Investigation Report (ISRA case no. E86611), and a Power Point presentation from March 16, 2010 entitled, Proposed Interim Specific Ground Water Quality Criteria and Interim Soil Remediation Standards – Johnson and Johnson Consumer Companies Inc., Former North Brunswick Facility. The first of these documents derives an Interim Ground Water Criterion for TCP of 280 µg/L based on the assertion of a NOAEL of 4 mg/kg/day in female rats from the NTP (1994) study of TCP. The second of these documents proposes an RfD of 0.04 mg/kg/day and an Interim Soil Remediation Standard of 2,444 ppm based on the same NOAEL.

Subsequently, the NTP (1994) study was reviewed. This review follows. As the review is not intended as a full RfD derivation for TCP, it does not summarize all aspects of the NTP study. Rather, it focuses on those aspects that relate to potential adverse effects of TCP from chronic exposure with a particular focus on toxicity associated with OPIDN.

A search of the toxicological scientific/toxicological literature subsequent to the NTP (1994) study did not reveal any additional original studies bearing on the toxicology of TCP mixtures.

Summary of Findings and Conclusions of the NTP (1994) TCP study

Test material

All testing was conducted on the same batch of TCP. Chemical analysis of that material showed it to be a complex mixture containing 79% mixed TCP and 18% mixed dicresyl phosphates. For the TCP, NTP identified 21% TMCP, 4% TPCP, 54% unidentified mixed TCP isomers. TOCP was not detected and NTP interpreted this to imply that TOCP, constituted <0.1% of the total. Note that the majority of the TCP was not further

characterized. In noting the non-detection of TOCP, NTP does not state that there is an absence of o-cresol-containing isomers, and subsequently discusses the possible role of such isomers in the observed toxicity of the mixture.

Results

Rats

13 wk gavage study

10 male and 10 female rats were dosed with 0, 50, 100, 200, 400, 800 mg/kg for 5 day/wk. This corresponds to nominal doses of 0, 36, 71, 142, 284, 568 mg/kg/day.

All animals survived through the study. Body weight in males was significantly reduced compared to controls for doses ≥142 mg/kg/day. No significant changes in body weight in females.

Relative liver weight was significantly increased in males at the highest dose and in females at ≥284 mg/kg/day. Relative thymus weight was significantly decreased in males at ≥100 mg/kg/day and females at ≥284 mg/kg/day.

Hematocrit, hemoglobin (Hb) concentration and erythrocyte density were significantly decreased in males at ≥284 mg/kg/day. In females, Hb were significantly decreased at 142 mg/kg/day. However NTP notes that the absolute magnitude of the decreases is small (for Hb at high dose, the decrease in males was 7.3%; and the decrease in females was 5.4%).

Cholinesterase activity in males and females was significantly reduced **at all doses.**

Hindlimb grip strength was significantly reduced in females at the two highest doses. However, NTP notes that the absolute magnitude of the reduction is small (13.7% at highest dose) and the standard deviation of controls is larger than that of high dose animals.

All animals (males and females) at all doses (but not controls) had vacuolization of cells in the adrenal cortex with an increasing severity score with increasing dose. Likewise for females for ovarian interstitial cell hypertrophy. In males, seminiferous tubule atrophy occurred at ≥284 mg/kg/day.

Based on decreased cholinesterase activity and decreased relative liver weight, we derive a LOAEL of 36 mg/kg/day for this portion of the study.

13 wk feed study

Rats were supplied feed with mixture concentrations of 0, 900, 1,700, 3,300, 6,600, and 13,000 ppm; corresponding to estimated doses of - males 55, 120, 220, 430 and 750 mg/kg/day; females 65, 120, 230, 430, 770 mg/kg/day.

All animals survived to termination of dosing. There was a significantly decreased body weight at ≥430 mg/kg/day in males and ≥230 mg/kg/day in females.

Relative liver weight was significantly increased in males and females at ≥120 mg/kg/day. Relative testes weight was significantly increased relative at ≥430 mg/kg/day.

There were no significantly hematological changes.

There was a significantly decreased in serum cholinesterase activity **at all doses** in males and females.

Enlargement of adrenal glands was noted in some males and females at ≥430 mg/kg/day. Adrenal cortex vacuolization was seen in males and females **at all doses**. Ovarian interstitial cell hypertrophy was also observed **at all doses** (9/10 at 65 mg/kg/day and 10/10 at all other doses). Ovarian chronic interstitial cell inflammation was observed **at all doses** (there were variable animals per dose level). In addition, various histopathological effects were noted in pituitary and kidneys at 220-430 mg/kg/day.

For this portion of the study, we derive a LOAEL based on decreased serum cholinesterase, enlarged adrenals and adrenal cortex histopathology is 55 mg/kg/day.

Two Year Feed Study

Animals were supplied feed with mixture concentration of 0, 75, 150, 300 ppm; corresponding to estimated doses of; males - 3, 6, 13 mg/kg/day and females - 4, 7, 15 mg/kg/day.

Survival of the dosed animals was similar to controls. Body weight for all doses in males and females was similar to controls. No clinical effects were seen at any doses.

At 15 months, serum cholinesterase levels in males were significantly decreased at 13 mg/kg/day and in females at 4 mg/kg/day.

Adrenal cortex cytoplasmic vacuolization in females at 15 mg/kg/day was observed at study termination (36/50 vs. 14/51 in controls). Ovarian interstitial hyperplasia was also observed at termination at 15 mg/kg/day.

We derive a LOAEL for the two year feed study in rats of 4 mg/kg/day for serum cholinesterase inhibition and 15 mg/kg/day for vacuolization of adrenal cortex and ovarian interstitial hyperplasia.

Mice

13-wk gavage study
Animals were dosed with 0, 50, 100, 200, 400, 800 mg/kg 5 day/wk, corresponding to nominal doses of 0, 36, 71, 143, 286, and 571 mg/kg/day.

All animals survived the duration of the dosing.
Body weight was significantly decreased in males at ≥143 mg/kg/day and in females at 286 mg/kg/day.

Males and females had a significantly increased startle response latency at ≥143 mg/kg/day. There was significantly decreased hindlimb grip strength in males at ≥143 mg/kg/day and in females at ≥286 mg/kg/day.

Liver weight was significantly increased in males at ≥286 mg/kg/day and in females at 143 mg/kg/day.

Significantly decreased cholinesterase activity occurred in males and females **at all doses.**

Significantly spinal cord and sciatic nerve axonal degeneration was observed in females at ≥71 mg/kg/day and in males at ≥143 mg/kg/day (with increasing severity score w. dose). **Note – This is a significant effect assoc. with OPIDN and strongly suggests the action of an o-cresol containing isomer.**

Significantly cytoplasmic vacuolization of the adrenal cortex was observed in males and females **at all doses**. In addition, significantly interstitial ovarian interstitial cell hypertrophy occurred in females **at all doses**.

We derive a LOAEL for this portion of the study is 36 mg/kg/day based on decreased serum cholinesterase activity, cytoplasmic vacuolization of the adrenal cortex and interstitial ovarian cell hypertrophy (This study did not produce a NOAEL).

13 wk feed study

Animals were given feed with concentrations of 0, 250, 500, 1,000, 2,100, 4,200 ppm corresponding to estimated doses of males – 45, 110, 180, 380, 900 mg/kg/day; females – 65, 130, 230, 530, 1,050 mg/kg/day

All animals survived to end of dosing. Significantly decreased body weight occurred in males at 900 mg/kg/day and in females at 530 mg/kg/day.

Forelimb grip strength was significantly decreased in males at ≥380 mg/kg/day and females at ≥530 mg/kg/day.

Relative liver weight was significantly increased at males at ≥380 mg/kg/day, and in females at ≥530 mg/kg/day **(NTP suggests these organ weight changes were due to decreased body weight)**.

Serum cholinesterase activity was significantly decreased in males and females **at all doses.**

There was a significantly increase in incidence of spinal cord and sciatic nerve axon degeneration at ≥530 mg/kg/day in females and 900 mg/kg/day in males. **As with the same effects in rats, this is closely associated with OPIDN and strongly suggests the presence of one or more o-cresol-containing isomers in the test mixture.**

There was s significantly increased incidence of cytoplasmic vacuolization in males at ≥110 mg/kg/day and in females **at all doses (≥65 mg/kg/day)**.

There was s significant incidence of ovarian interstitial cytoplasmic vacuolization at 530 mg/kg/day

Significantly incidence of gallbladder mucosal papillary hyperplasia was observed in males at 180 mg/kg/day and in females at 230 mg/kg/day.

We derive a LOAEL for this portion of the study of 45 mg/kg/day based on decreased serum cholinesterase activity.

2-Yr Feed Study

Animals were supplied feed containing the test material at 0, 60, 125, 250 ppm. This corresponds to estimated doses of: males – 7, 13, 27 mg/kg/day and females – 8, 18, 37 mg/kg/day

There was no significantly difference in survival compared to controls, and no significantly difference in body weight compared to controls.

No dose-related clinical effects were observed.

Significantly decreased serum cholinesterase activity occurred **at all doses** in males and females (≥7 and 8 mg/kg/day, respectively) at 15-mo interim evaluation.

Ceroid pigmentation of the adrenal cortex was observed at all doses and controls. However, there was a dose-related increase in severity. There was a significant increase in the incidence of liver cellular histopathology in males at 13 mg/kg/day.

There was a significantly increased incidence of adenomas of the Harderian gland at 27 mg/kg/day in males (5/50 vs. 0/52 in controls). **NTP states that the incidence in high dose males was within the range of historic controls.**

We derive a LOAEL for this portion of the study of 7 mg/kg/day based on decreased serum cholinesterase activity in males. (This study does not yield a NOAEL).

Genetic Toxicology

There was no induction of mutation in *Salmonella* test strains. No induction of sister chromatid exchange or chromosomal aberrations in Chinese hamster ovary cells, and no cell cycle delay.

Overall Summary of NTP studies

The effect occurring at the lowest level of exposure was decreased serum cholinesterase activity. This endpoint was seen consistently in both rats and mice and in each study. The LOAEL for this effect is 4 mg/kg/day in female rats in the 2-yr feed study. This was the lowest dose and there is, therefore no NOAEL.

Vacuolization of the adrenal cortex and ovarian cytoplasmic interstitial vacuolization were also seen consistently in rats and in mice with the exception of the 2-yr feed study.

Consideration of the appropriate RfD for TCP mixtures

No TOCP was detected in the analysis of the mixture used in the NTP (1994) study of TCP used throughout each of these tests (i.e., <0.1% of the total). However, the NTP mixture contained 54% uncharacterized TCP that was not further characterized with respect to the constituents of the mixed TCP isomers (i.e., isomers other than TOCP, TMCP and TPCP). In the ground water samples from the Johnson & Johnson site, TOCP was detected in a single sample of the ground water. A review of the analytical methodology employed by Accutest Laboratories (Dr. Lee Lippincott, NJDEP-Office of Science) reveals that the analytical methodology used in characterizing the isomers in the mixture was not capable of detecting mixed isomers that contained at least one o-cresol component. Mechanistic considerations indicate that a functional analog, the active toxicant of TOCP (the saligenin cyclic-o-tolyl phosphate (SCoTP)) can arise from a TCP isomer that contains only a single o-cresol constituent through the same steps that produce the SCoTP, the TOCP-based toxicant. Furthermore, Weiner and Jortner (1999) discuss the neurotoxicity of the mixed cresol TCPs containing one o-cresol, and the NTP (1994) study cites a 1958 study (in German) comparing the acute toxicity of TOCP in chickens to that of TCP isomers containing various combinations of m- and p-cresols with o-cresol(s). These data show that the presence of a single o-cresol with m- and/or p-cresols is capable of producing OPIDN and appears to result in greater OPIDN response than pure TOCP.

Unlike humans, rodents are not a sensitive species for production of OPIDN by TOCP (see assessment of TOCP – Dr. Alan Stern 11/16/10). Therefore, even if one or more

isomers of TCP in the NTP test mixture contained an o-cresol component, it would not be expected that OPIDN would have been observed. However, both axonal degeneration (spinal and sciatic) and serum acetylcholinesterase inhibition were observed. These are consistent with the neurotoxic effects of TOCP in rodents and axonal degeneration in particular, is closely associated with OPIDN in chickens. The observation of these effects suggests that one or more isomers containing an o-cresol and capable of producing the SCoTP-like functional analog of the TOCP toxicant was present in the NTP test mixture.

Therefore, although the LOAEL for the overall NTP study is 4 mg/kg/d, this may not qualitatively reflect the toxic potential for human exposure. Given the evidence suggesting that mixed isomers containing at least one o-cresol component can have at least as great an OPIDN potential as TOCP, it is prudent to apply the RfD derived for TOCP to the derivation of mixed TCP isomers rather than an RfD based on the LOAEL from the NTP study.

Based on the RfD and Interim Specific Ground Water Criterion previously recommended for TOCP (i.e., 4×10^{-4} mg/kg/day and 3 μg/L, respectively), it is recommended that the same RfD and Interim Specific Ground Water Criterion be applied to mixtures characterized as total TCP unless isomer-specific analyses are carried out and show no detectable o-cresol-containing isomers at an appropriate level of detection.

References

NTP (National Toxicology Program) (1994). Toxicology and carcinogenesis studies of tricresyl phosphate (CAS No. 1330-78-5) in F344/N rats and B6C3F_1 mice. US Department of Health and Human Services, Public Health Service. Accessed at: http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr433.pdf,

Weiner ML, Jortner BS (1999). Organophosphate-induced delayed neurotoxicity of triarylphosphates. Neurotoxicology. 20:653-73.