ORGANOTIN (Methyl and Butyl) TOXICITY

Occurrence

Tin (Sn) is a silver metal (atomic number 50) that occurs in the earth's crust mainly as cassiterite or tinstone (SnO2) which is the main source of tin production (*1*). The toxicity of inorganic tin after inhalation and ingestion is low. Absorbed tin is mostly stored in bone and excreted via urine (*1*). Tin is a trace element that is required in bone formation. Tin may occur in an inorganic or organic form. Some of the organotin compounds can be quite toxic causing neurotoxicity, hepatotoxicity, renal toxicity or dermal toxicity (*1*).

The organotins can be classified into two groups based on their use. These groups are the pesticidal tins, usually triorganocompounds, used as antifoulant paints, agricultural pesticides and molluscicides (*1*). The concentrations of tins in rivers, estuaries, lakes and oceans are generally less than 5 ng/L but levels as high as 3,300 ng/L have been reported in Lake Michigan (*1*).

The other use of tin is as a heat stabilizer in the production of PVC materials, curing agents for silicon rubber and catalysts in the production of polyurethane. The organotins find their way into the drinking water through this second pattern of use. One Canadian national survey found the concentrations of methyl and butyltins to range from 92-324 ng/L with monomethyltin being the highest and dimethyltin the lowest. The monobutyl and dibutyltins were at intermediate concentrations (cited in an EPA Office of Ground Water and Drinking Water preliminary assessment of organotin compounds, (Draft document, 2000) Karen Wirth, personal communication). In another Canadian survey, monomethyl-, dimethyl-, monobutyl and dibutyltin levels ranged up to 291 ng Sn/L, 49.1 ng Sn/L, 28.5 ng Sn/L, and 52.3 ng Sn/L, respectively (*2*).

The National Sanitation Foundation (NSF) has established standards that are nonregulatory and voluntary. The NSF action levels are 100 µg/L for short-term exposure to mono- and dimethyltin with 30 µg/L for chronic exposure. The NSF action levels are 100 µg/L and 30 µg/L for short term and chronic exposure to mono- and dibutyltin, respectively.

Toxicity of Organotins in humans

Workers handling dibutyl- and tributyltin have reported eye irritation and skin lesions (*1*) and mucus irritation after exposure to interior paints containing tin (*3*). Toxicity of organotins in humans is most frequently reported as loss of memory and insomnia as well as other symptoms including death (*4*). Neurotoxicity has also been reported with trimethyltin exposure in humans (*1*). Liver damage has been reported in people spraying triphenyltin acetate (*1*). In six workers exposed to a solution of 75% dimethyltin and 25 % trimethyltin for a total of 90 minutes over three days, one worker died, one remained hospitalized and only three were able to return to work (*4*). The toxicity of organotins is specific for the different organo- species of tin, for example triethyltin is myelinotoxic while trimethyltin is toxic to neurons of the limbic system (*5*). Different patterns and target tissues of toxicity may be predicted for the mono-and di- methyl- and butyltins found in the drinking water. For example, dibutyltin causes cholangiolar cell toxicity in rats. The toxicity generally tends to decrease with fewer organic groups on the tin.

Toxicity of Organotins in *in vitro* studies

For a series of diorganotins, the dibutyltin is the most cytotoxic for brain derived cells while the dimethyltin is the least toxic (*6*). Using the SOS chromotest, monobutyltin and dibutyltin showed genotoxicity while the mono- and dimethyltins were not genotoxic (*7*). Monobutyltin and dibutyltin were mutagenic in Salmonella typhimurium TA100 while only dibutyltin was positive in Salmonella typhimurium TA98 (*8*). In these assays, monomethyltin and dimethyltin failed to show mutagenicity (*8*).

Toxicity of Organotins in animal studies

In general there is a lack of rodent subchronic and chronic studies for the organotin compounds but developmental toxicity studies have been conducted for butyltins (*9*). Some short-term studies identify immunological (*10*) and nervous systems as being sensitive to organotins (*11*) with hepatic and renal effects being less frequently reported.

Methyltins

1) Methyltin (CH_3-Sn; CAS No. 16408-15-4) (Monomethyltin trichloride)

Pregnant rats were exposed to 12 and 120 mg/L monomethyltin in the drinking water during gestation. Acquisition and extinction learning ability were impaired in the pups compared to controls (*12*).

2) Dimethyltin (C_2H_6-Sn; CAS No. 23120-99-2) (Dimethyltin dichloride)

A 13 week dietary study in rats of a monomethyl (78.4%) dimethyl (21.6%) tin combination at 20, 100 and 500 ppm showed renal and urinary bladder changes primarily at the high concentration. This Dutch study was cited in an EPA Office of Ground Water and Drinking Water preliminary assessment of organotin compounds.

The data on monomethyl- and dimethyltin was considered to be inadequate by EPA for calculation of a RfD, (Karen Wirth, EPA, Office of Ground Water, personal communication, April 2000). EPA considered additional toxicology studies necessary for methyl- and dimethyltin.

The trimethyltins have been more widely studied and are neurotoxic. Human exposure to trimethyltin is usually an occupational risk (*11, 13*). Trimethyl exposure generally does not occur via drinking water.

Butyltins

1) Monobutyltin (C_4H_9-Sn; CAS No. 78763-54-9) (butyltin)

Pregnant rats were exposed to 50 and up to 400 mg/L monobutyltin by gavage on days 7 through 17 of gestation. There were no effects of monobutyltin on the dams or the fetuses compared to controls (*14*). Doses of 1,000, 1,500 or 2,000 mg/kg of butyltin were administered by gavage to Wistar rats on gestation days 7 and 8 with sacrifice on gestation day 20. All animals died in the top dose with weight loss in dams and fetuses in the other groups, but malformations were not found (*9*).

2) Dibutyltin (C_8H_2O-Sn; CAS No. 1002-53-5) (Di-n-butyltin)

Dibutyltin was found not to be carcinogenic in an NCI bioassay where animals were dosed up to 6.6 mg/kg bw/day rats and 19.8 mg/kg bw/day for 78 weeks for mice (*15*). Pregnant rats were exposed to 1.7 to 50 mg/kg/day dibutyltin diacetate by gavage on days 7 through 17 of gestation. Dibutyltin caused decreased thymic weights for all doses and decreased maternal weights for the highest dose. Increased fetal mortality and increased malformations were found compared to controls (*14*). Doses of 10 or 15 mg/kg of dibutyltin dichloride were administered by gavage to Wistar rats on gestation days 7 and 8 with sacrifice on gestation day 20. There were significant decreases in maternal weight gain, decreased fetal weights, higher postimplantation loss and increased fetal malformations compared to controls (*9*).

The data on monobutyl- and dibutyltin were considered to be inadequate by EPA for calculation of a RfD. Further toxicity studies were considered high priority for monobutyl- and dibutyltin (Karen Wirth, EPA, Office of Ground Water, personal communication, April 2000).

<u>Rationale for consideration of testing organotins in the drinking water</u>

The list of contaminants (drinking water Contaminant Candidate List, CCL) contains organotins as a class. Organotins have wide application (more than 35,000 tons in 1989 (*16*)) with more that 65% being used in PCV pipes. Organotins also find their way into surface waters from their use as pesticides.

Organotins (principally mono- and di- methyltins and butyltins) occur in the drinking water. The concentrations are in the low ng/L range. There is very limited data on the mono- and dibutyltins and essentially no data on mono- and dimethyltins. Toxicology data sets from the triethyl and trimethyltins are quite different suggesting that existing data sets would not be predictive.

Since mono- and dimethyltins and also mono- and dibutyltins tend to occur together in specific ratios, it would be practical and also defensible to evaluate the mono- and dimethyltins in one study and the mono- and dibutyltins in a second study.

There is clear evidence that the triethyltin and the trimethyltin are neurotoxic in experimental animals under certain conditions. There is inadequate evidence for the potential neurotoxic effect of chronic exposure to the organotin species that exist in the drinking water. It is important to include cognitive function in the neurotoxicological examination.

Proposed Studies

Both the methyl and butyltins are of interest to the EPA Office of Water for consideration for further studies. The butyltins appear more toxic and are found at similar levels to the methyltins in the drinking water. However, the methyltins appear to have a great potential to cause neurotoxicity because of the clear neurotoxicity caused by trimethyltin. Generally, the drinking water contains a combination of methyltins or the butyltins. They rarely occur together. The organotins may be developmental neurotoxicants. Therefore, we propose two types of short-term tests. The first would test the effect of a mixture of mono- and dimethyltin in 14-day study drinking water and a mixture of mono- and dibutyltin in a second 14-day drinking water study. The second type of test would be to evaluate the same mixtures in pregnant rats and mice with dosing to continue through lactation and until the animals are 4 to 5 weeks old. At this stage the pups would be evaluated for neurobehavioral effects. Histological examination would also be included. This should provide information on the potential developmental neurotoxicity potential of these organotins.

Single gavage doses of each of the 4 organotins may be necessary to establish toxicity and whether tin localizes in specific areas of the brain. Expected target tissues include the brain, liver and kidney. Long-term (6 to 12 months) comprehensive neurotoxicology examination with both behavioral and cognitive endpoints may be indicated. Based on the short-term studies either the mono- and dibutyltin combination or the mono- and dimethyltin combination will be selected for the long-term studies. The study may include only the F344 rats. However the B6C3F1 mice may be selected for long-term studies if the short-term studies provide compelling data.

Since cancer is not considered a high priority, the emphasis needs to be on neurobehavioral and cognitive function evaluation at multiple time points.

A detailed neuropathological examination of the nervous system will be conducted using both traditional stains and special stains for reactivity and neurodegeneration in the nervous system with a traditional examination of other organs and tissues.

Short-term studies and developmental studies should included animals for EPA NHEERL investigators to investigate sensitive indicators of neuronal damage and myelin. It is recommended that the dosing be done under NTP conditions with the animals or tissues provided to EPA investigators who will assist in the evaluation of the potential neurotoxicity of organotin species found in the drinking water.

Bone is a site of tin deposition and bone tin levels may contribute to our understanding of the body burdens between the methyltin and butyltin studies. Analysis of tin in both bone and brain should be considered following the long-term studies. Localization of tin to specific areas in the brain may be considered if neurological symptoms suggest specific areas may be affected.

There are several new transgenic mouse models for degenerative neuropathologies. This study offers the opportunity to evaluate the potential role of organotins in enhancing the onset of disease in these models. No one specific transgenic animal addresses the multiple targets for neurodegenerative diseases in humans. Therefore there is a need to use several transgenic mouse models that are reflective of the types of damage that can occur. An IL-6 transgenic model shown signs of neurodegeneration within 6 months. This model may allow exploration of the role of tin in inflammatory factors in the various neurodegenerative processes. This would shorten the time needed to study the disease process compared to the traditional rodent models.

The EPA Office of Water has also expressed interest in potential reproductive and developmental effects. Therefore organotin developmental reproductive toxicity studies also need to be considered.

References

1. Magos, L. Tin. In: *Handbook on the toxicology of metals* (2nd ed.), edited by L. Friberg, G. F. Nordberg and V. Vouk. New York: Elsevier, 1986, p. 568-593.
2. Sadiki, A.-I., and D. T. Williams. A study on organotin levels in Canadian waters distributed through PVC pipes. *Chemosphere* **38**, 1541-1548 (1999).
3. Wax, P. M., and L. Dockstader. Tributyltin use in interior paints: a continuing health hazard. *J. Toxicol. Clin. Toxicol.* **33**, 239-241 (1995).
4. Rey, C. H., H. J. Reinecke, and R. Besser. Methyltin intoxication in six men: Toxicologic and clinical aspects. *Vet. Hum. Toxicol.* **26**, 121-122 (1984).
5. Chang, L. W. The neurotoxicology and pathology or organomercury, organolead, and organotin. *J. Toxicol. Sci* **15; Suppl 4**, 125-151 (1990).
6. Borenfreund, E., and H. Babich. *In vitro* cytotoxicity of heavy metals, acrylamide, and organotin salts to neural cells and fibroblasts. *Cell Biology Toxicol.* **3**, 63-73 (1987).
7. Hamasaki, T., T. Sato, H. Nagase, and H. Kito. The genotoxicity of organotin compounds in SOS chromotest and rec-assay. *Mutat. Res.* **280**, 195-203 (1992).
8. Hamasaki, T., T. Sato, H. Nagase, and H. Kito. The mutagenicity of organotin compounds as environmental pollutants. *Mutat. Res.* **300**, 265-271 (1993).
9. Ema, M., R. Kurisaka, H. Asmano, and Y. Ogawa. Comparative developmental toxicity of butyltin trichloride, dibutyltin dichloride and tributyltin chloride in rats. *J. Appl. Toxicol.* **15**, 297-302 (1995).
10. Whalen, M. M., B. G. Loganathan, and K. Kannan. Immunotoxicity of environmentally relevant concentrations of butyltins on human natural killer cells *in vitro*. *Environmental Res.* **81**, 108-116 (1999).
11. Xiao, Y., G. J. Harry, and K. R. Pennypacker. Expression of AP-1 transcription factors in rat hippocampus and cerebellum after trimethyltin neurotoxicity. *Neurotoxicology* **20**, 761-766 (1999).
12. Norland, E. A., D. H. Taylor, and R. J. Bull. Monomethyltin and trimethyltin compounds induce learning deficiencies in young rats. *Neurobehav. Toxicol. Teratol.* **4**, 539-544 (1992).
13. Dorman, D. C. An integrative approach to neurotoxicity. *Toxicol. Pathol.* **28**, 37-42 (2000).

14. Noda, T., T. Yamano, M. Saitoh, T. Nakamura, and S. Morita. Comparative teratogenicity of di-n-butyltin diacetate with n-butyltin trichloride in rats. *Arch Environ. Contam. Toxicol.* **23**, 216-222 (1992).
15. NCI. Bioassay of dibutyltin diacetate for possible carcinogenicity. National Cancer Institute (NCI) Technical Report 183, DHEW Publication No. NIH 79-1739 Washington, DC,1979.
16. Sadiki, A.-I., D. T. Williams, R. Carrier, and B. Thomas. Pilot study on the contamination of drinking water by organotin compounds from PVC materials. *Chemosphere* **32**, 2389-2398 (1996).