Toxicity of 3,3',4,4'-tetrachloroazobenzene and 3,3',4,4'-tetrachloroazoxybenzene in rats and mice

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Introduction

3,3',4,4'-Tetrachloroazobenzene (TCAB) and 3,3',4,4'-tetrachloroazoxybenzene (TCAOB) are not commercially manufactured but are formed as unwanted byproducts in the manufacture of 3,4-dichloroaniline (DCA) and its herbicidal derivatives Propanil (TCAB only), Linuron (TCAB only), and Diuron[1,2)]. Both compounds have been shown to exhibit dioxin-like properties, including binding to the Ah receptor, induction of cytochrome P4501A1, and production of thymic atrophy[3-5)]. Although TCAB and TCAOB can be considered dioxin-like compounds, only limited toxicity studies have been performed in rats and mice. The present studies examined dose-response relationships for histopathological findings and changes in organ weights, clinical chemistry, hematology, and selected reproductive parameters. For a detailed description of both studies, see the technical reports of the National Toxicology Program[1,2)] and Van Birgelen *et al.*[6,7)].

Methods

Chemicals: TCAB and TCAOB were purchased from AccuStandard, Inc. (New Haven, CT) and were identified by infrared spectroscopy. Gas chromoatography indicated a purity of 98%.

Animals and treatment: Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic farms (Germantown, NY). Water and food were available *ad libitum*. Animals were randomly assigned to treatment groups. Male mice were housed individually and rats and female mice were housed five per cage. Groups of 10 male and 10 female rats and mice were administered 0, 0.1, 1, 3, 10, or 30 mg/kg TCAB or TCAOB in corn oil by gavage 5 days a week for 13 weeks. The total dosing volume was 10 mL/kg body wt for the mice and 5 mL/kg for the rats. Animals were observed twice a day for mortality and moribundity and weekly for clinical signs of toxicity. Individual animal weights were recorded weekly. At the end of the 13-week studies, animals were killed by CO_2 asphyxiation. Serum chemistry analyses were performed in rats and mice; hematology studies and thyroid hormone analyses were performed only in rats. The weights of the liver, thymus, right kidney, heart, spleen, right testis, uterus, and lung were determined at necropsy and 35+ tissues were taken for microscopic evaluations. Sperm morphology and characterization of the estrous cycle were analyzed in 10 rats and 10 mice treated with 0, 3, 10, or 30 mg TCAB/kg for 13 weeks, as described by Morrissey and co-workers[8)]. For the TCAOB studies, rats and mice exposed to 0, 1, 3, or 10 mg/kg and 0, 3, 10, or 30 mg/kg for 13 weeks were analyzed, respectively.

Results and discussion

Tables 1 and 2 summarize the main effects of TCAB and TCAOB respectively, in the 13-week gavage studies in both male and female rats and mice.

One of the most striking effects was a marked decrease in circulating thyroid hormone concentrations, whereas thyroid-stimulating hormone (TSH) was hardly affected (see table 3). In addition, TCAOB resulted in dilatation of keratin-containing hair follicles in mice, which resembled chloracne-like lesions. Although chloracne-like lesions have been observed before in other species exposed to dioxin-like compounds, this effect has never been observed in mice other than hairless or rhino mice[9]. Other dioxin-related effects included thymic atrophy, an increase in liver weights, and decreased mean body weight gains. A no-observable-adverse-effect-level (NOAEL) was not reached in rats for either compound. For TCAB the NOAEL in mice was 0.1 mg/kg. For TCAOB the NOAEL in male and female mice was 1 and 0.1 mg/kg, respectively.

Table 1. Summary of selected treatment-related effects in the 13-week gavage studies of TCAB in F344/N rats and B6C3F$_1$ mice.

	Affected dose group (mg/kg)			
Endpoint	Male Rats	Female Rats	Male Mice	Female Mice
Terminal body weight (decrease)	30	30	NS[a]	NS
Body weight gain (decrease)	NS	30	NS	NS
Liver				
Weight (increase)	3↑	10↑	10↑	10↑
Centrilobular hypertrophy of hepatocytes (increased incidence)	NO[b]	NO	3↑	NO
Thymus				
Weight (decrease)	10↑	10↑	30↑	NS
Atrophy (increased incidence)	10↑	30	NO	NO
Spleen				
Weight (increase)	10↑	30	30	10↑
Hematopoietic cell proliferation (increased incidence)	10↑	10↑	3↑	NS
Responsive anemia	10↑	10↑	-[c]	-
Platelet count (decrease)	10↑	10↑	-	-
Total T3 and T4 concentrations[d] (decrease)	0.1↑[e]	0.1↑[e]	-	-
Forestomach				
Epithelial hyperplasia (increased incidence)	3 and 30	30	1, 10 and 30	1↑
Epididymal spermatozoal concentration (decrease)	NS	-	3[e] and 30	-

↑ All higher doses were affected
a) NS = Not significantly affected
b) NO = Not observed
c) Not applicable or not analyzed
d) T3 = triiodothyronine; T4 = thyroxine
e) Lowest dose tested for this effect

Table 2. Summary of selected treatment-related effects in the 13-week gavage studies of TCAOB in F344/N rats and B6C3F$_1$ mice.

	Affected dose group (mg/kg)			
Endpoint	Male Rats	Female Rats	Male Mice	Female Mice
Deaths	30	30	NO[a]	NO
Terminal body weight (decrease)	3↑	10↑	NS[b]	NS
Body weight gain (decrease)	3↑	10↑	NS	NS
Lung				
Weight (increase)	NO	10↑	NO	NO
Chronic active inflammation of vasculature (increased incidence)	10↑	30	NO	NO
Heart				
Weight (increase)	NO	30	NO	NO
Severity of cardiomyopathy (increased severity)	30	30	NO	NO
Skin				
Dilatation of hair follicles (increased incidence)	-[c]	-	10↑	30
Liver				
Weight (increase)	1↑	3↑	3↑	1↑
Centrilobular hypertrophy of hepatocytes (increased incidence)	NO	NO	10↑	10↑
Hematopoietic cell proliferation in liver (increased incidence)	30	30	NO	NO
Centrilobular degeneration of hepatocytes (increased incidence)	10↑	30	NO	NO
Thymus				
Weight (decrease)	1↑	1↑	3↑	10↑
Atrophy (increased incidence)	3↑	10↑	NS	NS
Kidney				
Weight (increase)	NO	30	NS	10↑
Nephropathy (increased incidence)	NS	3↑	NO	-
Forestomach				
Hyperplasia (increased incidence)	3↑	10↑	10↑	30
Spleen				
Weight (increase)	NO	10↑	NO	NO
Hematopoietic cell proliferation (increased incidence)	30	10↑	30	10↑
Responsive anemia	1↑	10↑	-	-
Platelet counts (decrease)	3↑	0.1↑	-	-
Total T3 concentration[d] (decrease)	NS	10↑	-	-
Total T4 concentration[e] (decrease)	1↑	0.1↑	-	-
Epididymal spermatozoal motility (decrease)	1, 3, and 10[f]	-	NO	-
Estrous cycle length (longer)	-	10	-	NO

↑ All higher doses were affected
a) NO = Not observed
b) NS = Not significantly affected
c) Not applicable or not analyzed
d) T3 = triiodothyronine
e) T4 = thyroxine
f) Only doses tested for this effect

Table 3. Thyroid hormone concentrations in 13-wk gavage studies of TCAB and TCAOB in F344/N rats.

Parameter	Vehicle control	0.1 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Male rats-TCAB[a]						
TT4 (µg/dL)	3.4 ± 0.2[b]	2.1 ± 0.2*	0.6 ± 0.2*	0.5 ± 0.1*	0.5 ± 0.1*	0.1 ± 0.1*
TT3 (ng/dL)	140 ± 8	113 ± 3*	121 ± 6*	116 ± 6*	119 ± 4*	102 ± 6*
TSH (ng/mL)	2.0 ± 0.3	1.8 ± 0.2	1.9 ± 0.2	2.3 ± 0.3	2.7 ± 0.3	3.4 ± 0.5*
Female rats-TCAB						
TT4 (µg/dL)	2.3 ± 0.2	1.7 ± 0.2*	0.5 ± 0.1*	0.2 ± 0.0*	0.1 ± 0.0*	0.0 ± 0.0*
TT3 (ng/dL)	131 ± 5	112 ± 5*	107 ± 6*	102 ± 5*	101 ± 5*	99 ± 6*
TSH (ng/mL)	1.0 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.4 ± 0.2
Male rats-TCAOB						
TT4 (µg/dL)	2.9 ± 0.1	3.0 ± 0.1	1.9 ± 0.1*	0.9 ± 0.1*	0.4 ± 0.1*	-[c]
TT3 (ng/dL)	98 ± 5	118 ± 7	102 ± 6	117 ± 8	99 ± 7	-
TSH (ng/mL)	1.6 ± 0.2	2.6 ± 0.2*	2.9 ± 0.5*	3.1 ± 0.5*	3.5 ± 0.6*	-
Female rats-TCAOB	2.5 ± 0.2	1.7 ± 0.2*	1.2 ± 0.1*	0.3 ± 0.1*	0.1 ± 0.0*	0.0 ± 0.0*
TT4 (µg/dL)	97 ± 6	90 ± 7	89 ± 5	83 ± 3	64 ± 4*	83 ± 6*
TT3 (ng/dL)	1.1 ± 0.2	0.9 ± 0.1	1.1 ± 0.2	1.2 ± 0.2	1.5 ± 0.2	1.4 ± 0.2
TSH (ng/mL)						

*Significantly different ($p<0.05$) from the vehicle control group by Dunn's or Shirley's test.
a) TT4 = total thyroxine; TT3 = total triiodothyronine; TSH = thyroid-stimulating hormone
b) Mean ± SE.
c) No data available due to 100% mortality.

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