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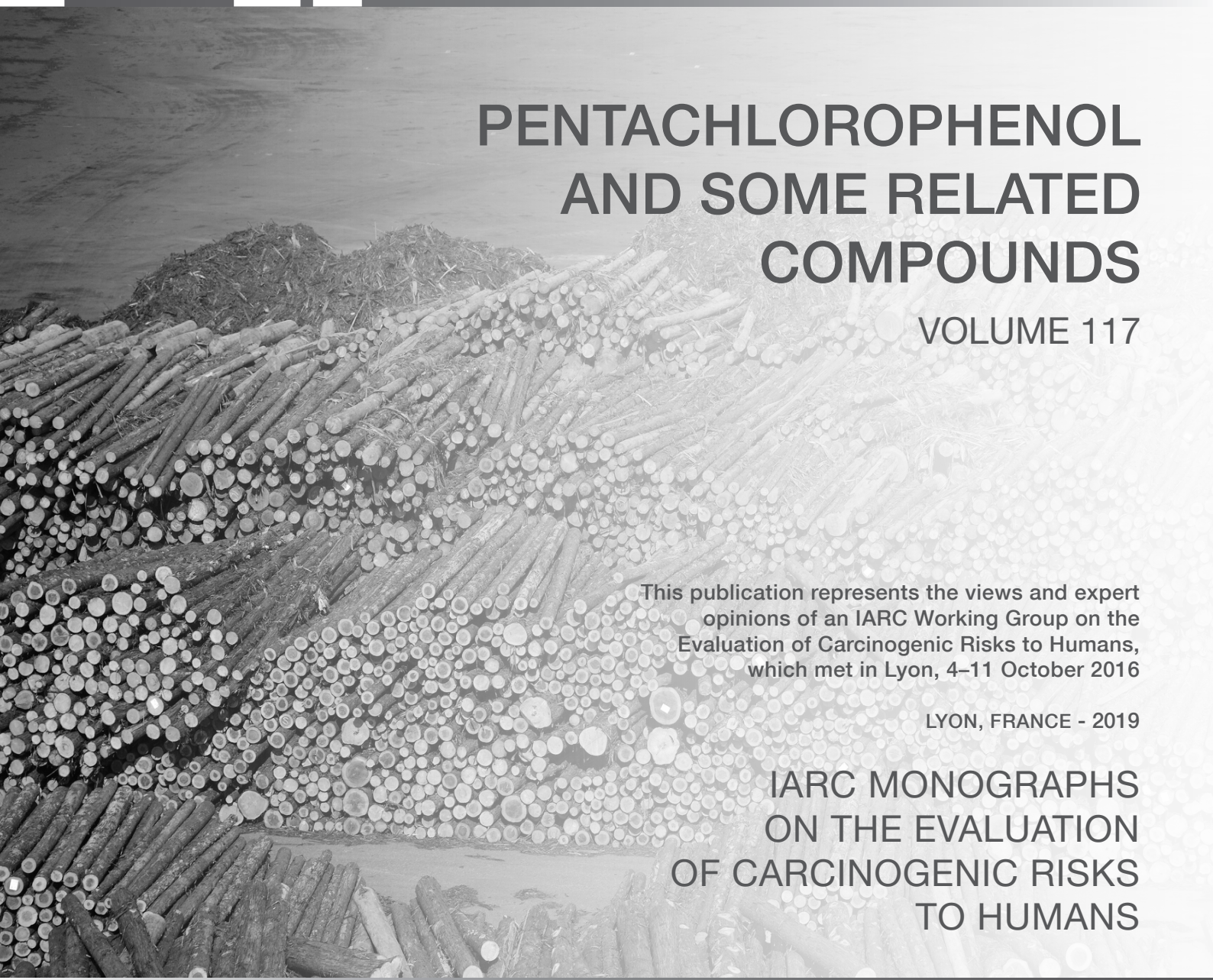

PENTACHLOROPHENOL AND SOME RELATED COMPOUNDS

VOLUME 117

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International Agency for Research on Cancer

World Health Organization

3,3′,4,4′-TETRACHLOROAZOBENZENE

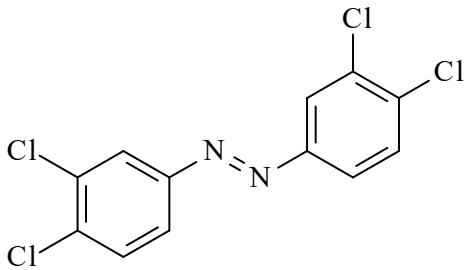
1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 14047-09-7

Chem. Abstr. Serv. Name: 3,4,3′,4′-Tetrachloroazobenzene

Synonyms: Bis(3,4-dichlorophenyl)diazene; azobenzene, 3,3′,4,4′-tetrachloro-; diazene, bis(3,4-dichlorophenyl)-; diazene, bis(3,4-dichlorophenyl)- (9Cl); TCAB



Molecular formula: $C_{12}H_6Cl_4N_2$

Relative molecular mass: 320

In the *trans* configuration, 3,3′,4,4′-tetrachloroazobenzene (TCAB) can assume a planar conformation with a molecular shape similar to that of 2,3,7,8-tetra-chlorodibenzo-*para*-dioxin (TCDD) (Poland et al., 1976; NTP, 2010)

Description: Bright orange, crystalline solid

Melting point: 158 °C

Solubility: water solubility: 6.72×10^{-3} mg/L at 25 °C

Vapour pressure: 1.56×10^{-7} mm Hg (2.07×10^{-5} Pa) at 25 °C

Hazardous decomposition: When heated to decomposition it emits toxic fumes of chlorine and oxides of nitrogen

Octanol/water partition coefficient: log K_{ow}, 5.53 (Hashimoto et al., 1994; NTP, 1998)

Stability: TCAB is stable as a bulk chemical when stored at room temperature (NTP, 2010)

Conversion factor: 1 ppm = 13.1 mg/m^{3,} at normal temperature (25 °C) and pressure (1 atm).

1.2 Production and use

1.2.1 Production

TCAB is not commercially manufactured but is formed as an unwanted by-product in the manufacture of 3,4-dichloroaniline and its herbicidal derivatives, which include propanil (3′,4′-dichlorophenylpropionanilide; CAS No., 709-98-8), linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea; CAS No., 330-55-2), diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea; CAS No., 330-54-1), and neburon (1-butyl-3-(3,4-dichlorophenyl)-1-methylurea; CAS No., 555-37-3) (Poland et al., 1976; Sundström et al., 1978; Bunce et al., 1979; Hill et al., 1981).

In the late 1980s in the USA, when the production volume of propanil was 10 million pounds [~4536 tonnes] per year, the resultant annual production of TCAB for propanil alone may have been as high as 12 000 kg (McMillan et al., 1991). Likewise, with a production volume of 100 000–1 000 000 pounds [45–454 tonnes] of 3,4-dichloroaniline per year, the resultant annual production of TCAB may have been as high as 3900 kg (EPA, 1985). Because 3,4-dichloroaniline is used as a precursor in dye manufacture and, to a limited extent, as a heat transfer fluid, in addition to its use in the manufacture of herbicides (EPA, 1985), TCAB could be present in products other than herbicides (NTP, 2010).

1.2.2 Use

No known direct use of TCAB has been reported.

In 2007, the use of propanil and diuron in the USA was estimated to range from 4 million to 6 million pounds [1814–2722 tonnes] and from 2 million to 4 million pounds [907–1814 tonnes], respectively (EPA, 2011).

In California, USA, the use of several anilide pesticides including propanil was reported to have increased from < 1000 pounds in 1998 to ~2 million pounds [< 0.45 to ~907 tonnes] in 2014 (OEHHA, 2016).

1.3 Methods of analysis

No data were available to the Working Group.

1.4 Occurrence and exposure

Concentrations of TCAB in technical grades ranged from 0.1 to 9.9 µg/g for propanil, 5.7 to 12 µg/g for diuron, 6.7 to 28 µg/g for linuron, and 1.9 to 23 µg/g for neburon (Di Muccio et al., 1984). Hill et al. (1981) found TCAB at concentrations of 1000–1400 µg/g in propanil, 9–51 µg/g in 3,4-dichloroaniline, 28 µg/g in diuron, and 9 µg/g in linuron; no detectable TCAB was reported in 1,2-dichlorobenzene or neburon (Hill et al. 1981). Singh & Bingley (1991) found TCAB at concentrations of 1–30 µg/g in commercial herbicides containing propanil (Singh & Bingley, 1991). Call et al. (1983) found TCAB at a concentration of 670 µg/g in technical-grade propanil (Call et al., 1983).

In addition, environmental contamination by TCAB occurs from the degradation of chloroanilide herbicides (acylanilides, phenylcarbamates, and phenylureas) in soil by peroxide-producing microorganisms (Bartha et al., 1968; Bartha & Pramer, 1969; Lay & Ilnicki, 1974). TCAB is also formed by the photolysis and biolysis of 3,4-dichloroaniline (Miller et al., 1980; NTP, 2010).

Workers who manufacture or work with other products that have 3,4-dichloroaniline as a precursor (e.g. dyes) or as a heat transfer fluid may also be exposed to TCAB (EPA, 1985; NTP, 2010).

1.4.1 Occupational exposure

No measurements of TCAB exposure in workers were available to the Working Group. Occupational exposure may occur in workers involved in the manufacture of aniline herbicides, applicators who spray or mix aniline herbicide-containing formulations, and farm workers engaged in re-entry tasks. TCAB exposure would vary depending on the aniline herbicides used or produced (see Section 1.2).

1.4.2 Community exposure

The general population may be exposed to TCAB from residues on food, or from living near areas where aniline herbicides are applied.

(a) Sediment and soil

TCAB sorbs very strongly to soils, and has been detected in the top 10 cm of soil up to 2 years after application of propanil (Kearney et al., 1970). TCAB was found in 6 of 99 soil samples from rice-growing areas of the USA, with concentrations ranging from 0.01 to 0.05 ppm (Kearney et al., 1970; Carey et al., 1980). TCAB formation in soil varies with pH, with negligible formation in soils that are more alkaline than pH 6.0, and measurable levels in soils with a pH range of 4.0 to 5.5 (Hughes & Corke, 1974).

(b) Food

No data were available on TCAB measurements in the food supply; however, TCAB uptake in the food chain was observed experimentally in non-mammalian systems. Proportional increases in TCAB body burden were seen in Japanese medaka (*Oryzias latipes*) exposed to diets containing increasing concentrations of TCAB (0.5–2500 ppm) (Allinson & Morita, 1995a). The aquatic snail Indohiramakigai (*Indoplanorbis exustus*) was found to bioaccumulate TCAB from its environment during controlled exposures (Allinson & Morita, 1995b). Uptake of TCAB was also observed experimentally with soybeans (Worobey, 1984), carrots (Worobey, 1988), and rice plants (Still, 1969).

(c) Air, water, and residential dust

No data were available to the Working Group.

(d) Biological markers

No data on concentrations of TCAB in the general population were available to the Working Group.

1.5. Regulations and guidelines

In July 2012, California, USA, listed TCAB as a known carcinogen under the Safe Drinking Water and Toxic Enforcement Act (Proposition 65), based on findings from the National Toxicology Program (NTP) (OEHHA, 2012).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1.

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 5–6 weeks) were given TCAB (purity, ≥ 99.8%) at doses of 0 (control), 3, 10, or 30 mg/kg body weight (bw), in corn oil:acetone (99:1) by gavage, 5 days per week for 104 weeks (NTP, 2010). Survival was significantly decreased in males at 10 and 30 mg/kg bw, and in females at 30 mg/kg bw. At 0, 3, 10, and 30 mg/kg bw, survival was 35/50, 31/50, 5/50, and 0/50 in males, and 35/50, 30/50, 32/50, and 20/50 in females, respectively. Mean body weights of males at 10 and 30 mg/kg bw were 10% and 8% less than those of the vehicle controls at the last weighing at weeks 101 and 73, respectively. Mean body weights of females at 3 mg/kg bw were 7% greater than those of the vehicle controls after week 64.

The incidence of transitional cell carcinoma of the urethra was significantly increased (with a significant positive trend) in all treated groups of males. Two such neoplasms were also observed in females at 30 mg/kg bw (2/50; 4%). One male at 10 mg/kg bw and one female at 30 mg/kg bw had transitional cell carcinoma of the ureter. There was a significantly increased incidence of bronchioloalveolar adenoma of the lung in all treated groups of males, with a significant positive trend, and a significantly increased incidence of bronchioloalveolar carcinoma of the lung in females at 30 mg/kg bw, with a significant positive trend.

Table 3.1 Studies of carcinogenicity with 3,3′,4,4′-tetrachloroazobenzene (TCAB) in experimental animals

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 5–6 wk 104 wk NTP (2010)	Gavage TCAB, ≥ 99.8% Corn oil:acetone (99:1) 0, 3, 10, 30 mg/kg bw 10 mL/kg, 5 d/wk 50, 50, 50, 50 35, 31, 5, 0	*Urethra* Transitional cell carcinoma: 0/50*, 32/50**, 46/49**, 49/50**	*P (trend) < 0.001, **P < 0.001; poly-3 test	Significant decrease in survival at 10 and 30 mg/kg bw Principal strengths: the duration of exposure and observation was adequate, as was the schedule of exposure; GLP study Historical control incidence for bronchioloalveolar carcinoma (corn-oil gavage studies): 13/200 (6.5 ± 2.5%); range, 4–10%
		Lung Bronchioloalveolar adenoma: 5/50*, 16/50**, 12/49***, 6/50****	*P (trend) = 0.014, **P = 0.002, ***P = 0.006, ****P = 0.037; poly-3 test	
		Bronchioloalveolar adenoma or carcinoma (combined): 7/50*, 17/50**, 15/49***, 6/50	*P (trend) = 0.014, **P = 0.007, ***P = 0.003; poly-3 test	
		Bronchioloalveolar carcinoma: 3/50, 1/50, 4/49, 0/50	NS	
		Forestomach Squamous cell carcinoma: 0/50*, 1/50, 1/49, 3/50**	*P (trend) = 0.012, **P = 0.023; poly-3 test	
Full carcinogenicity Mouse, B6C3F$_1$ (F) 5–6 wk 105 wk NTP (2010)	Gavage TCAB, ≥ 99.8% Corn oil:acetone (99:1) 0, 3, 10, 30 mg/kg 10 mL/kg, 5 d/wk 50, 50, 50, 50 35, 30, 32, 20	*Urethra* Transitional cell carcinoma: 0/50, 0/50, 0/50, 2/50	NS	Significant decrease in survival at 30 mg/kg bw Principal strengths: the duration of exposure and observation was adequate, as was the schedule of exposure; GLP study Historical control incidence for cystic keratinizing epithelioma was 0/196 for corn-oil gavage studies and 0/1496 for all routes
		Lung Bronchioloalveolar carcinoma: 0/49*, 2/50, 1/50, 4/50**	*P (trend) = 0.031,**P = 0.042; poly-3 test	
		Bronchioloalveolar adenoma or carcinoma (combined): 3/49*, 8/50, 5/50, 10/50**	*P (trend) = 0.028, **P = 0.015; poly-3 test	
		Cystic keratinizing epithelioma: 0/49, 0/50, 0/50, 2/50	NR	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 5–6 wk 105 wk NTP (2010) (cont.)		*Forestomach* Squamous cell carcinoma: 0/50*, 1/50, 1/50, 4/50**	*P (trend) = 0.011, **P = 0.040; poly-3 test	
		Skin Fibrosarcoma: 1/50*, 6/50, 5/50, 8/50**	*P (trend) = 0.023, **P = 0.008; poly-3 test	
		Fibrosarcoma or malignant schwannoma: 2/50*, 8/50, 7/50, 12/50**	*P (trend) = 0.004, **P = 0.001; poly-3 test	
		Spleen/lymphatic tissue Malignant lymphoma: 2/50, 5/50, 8/50*, 7/50**	*P = 0.049, **P = 0.050; poly-3 test	
Full carcinogenicity Rat, Harlan Sprague-Dawley (M) 5 wk 104 wk NTP (2010)	Gavage TCAB, ≥ 99.8% Corn oil:acetone (99:1) 0, 10, 30, 100 mg/kg 2.5 mL/kg, 5 d/wk 50, 50, 50, 50 28, 9, 4, 2	*Lung* Cystic keratinizing epithelioma: 0/50*, 14/50**, 31/50**, 37/50**	*P (trend) < 0.001, **P < 0.001; poly-3 test	Significant decrease in survival in all treated groups Principal strengths: the duration of exposure and observation was adequate; as was the schedule of exposure; GLP study
		Oral mucosa Gingival squamous cell carcinoma: 1/50, 5/50*, 4/50, 5/50**	*P = 0.046, **P = 0.033; poly-3 test	
		Liver Cholangiocarcinoma: 0/50*, 4/50**, 4/50***, 6/50****	*P (trend) = 0.007, **P = 0.030, ***P = 0.026, ****P = 0.003; poly-3 test	
		Thyroid Follicular cell adenoma: 0/50*, 3/50, 4/50**, 4/50***	*P (trend) = 0.037, **P = 0.025, ***P = 0.021; poly-3 test	
		All organs Malignant schwannoma: 0/50*, 0/50, 1/50, 3/50	*P (trend) = 0.010; poly-3 test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Harlan Sprague-Dawley (F) 5 wk 104 wk NTP (2010)	Gavage TCAB, ≥ 99.8% Corn oil:acetone (99:1) 0, 10, 30, 100 mg/kg 2.5 mL/kg, 5 d/wk 50, 50, 50, 50 25, 30, 18, 17	*Lung* Cystic keratinizing epithelioma: 0/50*, 6/50**, 26/49***, 39/49***	* P (trend) < 0.001, **P = 0.014; poly-3 test, ***P < 0.001	Principal strengths: the duration of exposure and observation was adequate, as was the schedule of exposure; GLP study Historical control incidence for cholangiocarcinoma (corn-oil gavage studies): 1/473 (0.2 ± 0.7%); range, 0–2% Historical control incidence for gingival squamous cell carcinoma: 4/473 (0.8 ± 1.0%); range, 0–2% Historical control incidence for forestomach squamous cell papilloma: 0/473; forestomach squamous cell carcinoma: 2/473 (0.4 ± 0.8%) [range, 0–2%]
		Liver Cholangiocarcinoma: 1/50, 1/50, 1/49, 3/49	NS	
		Oral mucosa Gingival squamous cell carcinoma: 0/50*, 0/50, 4/50, 6/50**	*P (trend) = 0.002, **P = 0.015; poly-3 test	
		Forestomach Squamous cell papilloma: 0/50, 0/50, 0/50, 3/50	NS	
		Squamous cell carcinoma: 0/50, 1/50, 0/50, 1/50	NS	
		Squamous cell papilloma or squamous cell carcinoma (combined): 0/50*, 1/50, 0/50, 4/50	*P (trend) = 0.009; poly-3 test	

* Significance is indicated using asterisks

d, day(s); F, female; GLP, Good Laboratory Practice; M, male; NR, not reported; NS, not significant; TCAB, 3,3′,4,4′-tetrachloroazobenzene; wk, week(s)

A significantly increased incidence of bronchioloalveolar adenoma or carcinoma (combined) was observed in males at 3 and 10 mg/kg bw, and in females at 30 mg/kg bw. However, the incidence of bronchioloalveolar carcinoma was not increased in treated males compared with concurrent controls, or compared with the range for historical controls for corn-oil gavage studies for this neoplasm (incidence, 13/200; range, 4–10%).

In the forestomach, the incidence of squamous cell carcinoma in males and females at 30 mg/kg bw was significantly increased (3/50 and 4/50, respectively), with a significant positive trend, compared with that in the control groups receiving vehicle only (0/50 in males and females).

In females, the incidence of malignant lymphoma was significantly increased at 10 and 30 mg/kg bw. The incidence of fibrosarcoma, and of fibrosarcoma or malignant schwannoma (combined) of the skin was significantly increased in females at 30 mg/kg bw, with a significant positive trend. One occurrence of a single cystic keratinizing epithelioma and one occurrence of multiple cystic keratinizing epithelioma of the lung were reported in females at 30 mg/kg bw (2/50); the incidence of cystic keratinizing epithelioma in historical controls was 0/196 for corn-oil gavage studies and 0/1496 for all routes.

[The Working Group noted that in this study that complied with good laboratory practice (GLP), the duration of exposure and observation, and the schedule of exposure, were adequate.]

3.2 Rat

Groups of 50 male and 50 female Harlan Sprague-Dawley rats (age, 5 weeks) were given TCAB (purity, ≥ 99.8%) at doses of 0 (control), 10, 30, or 100 mg/kg bw, in corn oil:acetone (99:1) by gavage, 5 days per week, for 104 weeks (NTP, 2010). Survival of all treated groups of males (9/50, 4/50, 2/50) was significantly less than that of the controls receiving vehicle only (28/50). The number of females surviving to study termination was 30/50, 18/50, and 17/50 in the treated groups, respectively, compared with controls receiving vehicle only (25/50). Mean body weights of males at 100 mg/kg bw were less than those of males in the vehicle-control group throughout the study. Mean body weights of males at 30 mg/kg bw were 6% less than those of males in the vehicle-control group after week 24, and those of males at 10 mg/kg bw were 7% less than those of males in the vehicle-control group after week 80. Mean body weights of females at 100 mg/kg bw were less than those of females in the vehicle-control group throughout the study, and those of females at 30 mg/kg bw were 6% less than those of females in the vehicle-control group after week 36.

The incidence of multiple cystic keratinizing epithelioma and of cystic keratinizing epithelioma (including multiple) of the lung in males and females was significantly increased, with a positive trend, in all treated groups compared with controls, except for multiple cystic keratinizing epithelioma in females at 10 mg/kg bw.

In males, the incidence of cholangiocarcinoma of the liver in all treated groups was significantly greater than that in the control group, with a positive trend. In females, the incidence of cholangiocarcinoma of the liver in the group at the highest dose (3/49) was above the upper bound of the range for historical controls (incidence, 1/473; range, 0–2%).

The incidence of gingival squamous cell carcinoma of the oral mucosa was increased in treated males and females compared with controls: the increases in males at 10 and 100 mg/kg bw and in females at 100 mg/kg bw were significantly greater than those in the controls, and the increase in females at 30 mg/kg bw (6/50) exceeded the upper bound of the range for historical controls (incidence, 4/473; range, 0–2%), with a significant

positive trend in the incidence of this tumour in females.

There was a significant increase in the incidence of follicular cell adenoma of the thyroid gland in males at 30 or 100 mg/kg bw, with a significant positive trend.

There was a significant positive trend in the incidence of forestomach squamous cell papilloma or carcinoma (combined) in females. Two single and one multiple squamous cell papilloma of the forestomach occurred in females at 100 mg/kg bw; the incidence of this tumour in historical controls in females treated by gavage (corn oil) was 0/473. Single instances of forestomach squamous cell carcinoma occurred in males and females at 10 mg/kg bw, and in females at 100 mg/kg bw; the incidence of this tumour in historical controls in females was 2/473 (range, 0–2%).

In males, there was a significant positive trend in the incidence of malignant schwannoma in the thoracic cavity, with an incidence of 0/50 (control), 0/50, 1/50, and 3/50, respectively.

[In this GLP study, the duration of exposure and observation, and the schedule of exposure, were adequate.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Introduction

TCAB is a halogenated aryl hydrocarbon that is isosteric to TCDD, and is highly lipophilic. Like TCDD, TCAB binds to the aryl hydrocarbon receptor (AhR) and is a potent inducer of hepatic aryl hydrocarbon hydroxylase. In contrast to TCDD, however, TCAB is readily eliminated from the body (Pillai et al., 1996). TCAB is metabolized more readily than TCDD, and therefore does not bioaccumulate; however, the metabolic products of TCAB have structural alerts that suggest potential toxicity. Several studies have examined the toxicokinetics of TCAB in rats (Burant & Hsia, 1984; Pillai et al., 1996; NTP, 2010), TCAB is readily metabolized and two studies permit a biotransformation pathway to be compiled from the available data (Hsia & Kreamer, 1981; Pillai et al., 1996).

4.1.2 Absorption

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Studies in non-human mammalian models indicated that TCAB is absorbed either by inhalation or by dermal routes. For example, inhalation by rats (strain not specified), and dermal application in male albino rabbits, resulted in various systemic toxic effects of TCAB, indicating absorption (EPA OTS, 1983; EPA, 1985).

Several studies in rodents have shown that TCAB is readily absorbed via the oral route (Burant & Hsia, 1984; Pillai et al., 1996; NTP, 2010). In male Sprague-Dawley rats treated with [^{14}C]-labelled TCAB (single dose, 10 mg/rat) by gavage, a substantial amount of radiolabel (66% of the administered dose) was excreted in the urine and faeces after 24 hours, and a marked distribution of radiolabel into adipose tissue was found (Burant & Hsia, 1984). The oral bioavailability of TCAB (32 mg/kg) in male Fischer rats was calculated to be 30% when blood concentration–time curve (AUC) values were compared after oral and intravenous dosing regimens (Pillai et al., 1996). After oral administration, extensive azo reduction of TCAB (presumably by gut microbes) and first-pass metabolism may contribute to reduced systemic absorption, limiting the amount of TCAB that reaches the systemic circulation (Pillai et al., 1996). When TCAB was given orally to male Fischer rats as a

single dose (32 mg/kg bw), the apparent first-order absorption rate constant (K_a) and lag time for absorption (T_{lag}) were estimated to be 0.44 hour^{-1} and 1.5 hour, respectively (Pillai et al., 1996). By the end of a 3-month oral dosing regimen in female Sprague-Dawley rats (dose levels, 0, 0.1, 3, or 100 mg/kg bw), high concentrations of TCAB were found in the blood and tissues, with the highest amount found in adipose tissue (NTP, 2010). Together, these findings indicated that TCAB is absorbed via the gastrointestinal tract and widely distributed in experimental animals.

4.1.3 Distribution

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

The distribution of TCAB in rats has been evaluated in acute and chronic studies. In female Sprague-Dawley rats given TCAB as a single intravenous dose (2.87 mg/kg bw), half-lives of elimination ($t_{1/2\alpha}$ and $t_{1/2\beta}$) from the blood were 1 hour and 5.6 hours, respectively (NTP, 2010). The volume of distribution (V_{ss}) was 743 mL/kg bw, systemic clearance (CL_s) was 352 mL/hour per kg bw, and mean residence time (*MRT*) was 2.1 hours. [The Working Group noted that these data indicate that TCAB is rapidly distributed to tissues.] The terminal elimination half-life of ~6 hours indicated that TCAB is cleared relatively rapidly from the blood. Furthermore, prior exposure to TCAB (daily gavage doses of 3 mg/kg bw for 7 days) did not affect the distribution and elimination kinetics of TCAB.

Pillai et al. (1996) reported on the tissue distribution of [^{14}C]-labelled TCAB in male Fischer rats given single doses orally (3.2 and 32 mg/kg bw) or intravenously (3.2 mg/kg bw). Only 6% of the administered radiolabel remained in tissues 96 hours after dosing. Tissue distribution was similar after either oral or intravenous administration of TCAB. Adipose tissue exhibited by far the highest tissue-to-blood ratio, followed by kidney, with brain exhibiting the lowest ratio.

Distribution in rats after long-term exposure was also reported (NTP, 2010). TCAB levels in blood and tissue were monitored after the last dose of a 3-month study of TCAB (0.1, 3, or 100 mg/kg bw, by gavage) in female Sprague-Dawley rats. TCAB was mostly undetectable in blood of rats at the lowest dose. For the groups at 3 and 100 mg/kg bw, respectively, C_{max} was 192.3 and 619.8 ng/mL, and dose-normalized AUC values in blood were 332.8 and 28.7 ng•hour•kg/mL per mg, indicating a decrease in the relative amount of absorbed TCAB with increasing dose. Concentrations in adipose tissue were ~100 times those in liver and lung, and gradually declined, with half-lives in adipose tissue of 115, 81, and 86 hours for the groups at 0.1, 3, and 100 mg/kg bw, respectively. In general, similar half-lives were found in liver and lung. After a 3-month exposure, TCAB was mainly distributed to adipose tissue, where it was moderately persistent, whereas the extent of distribution to other tissues was more limited.

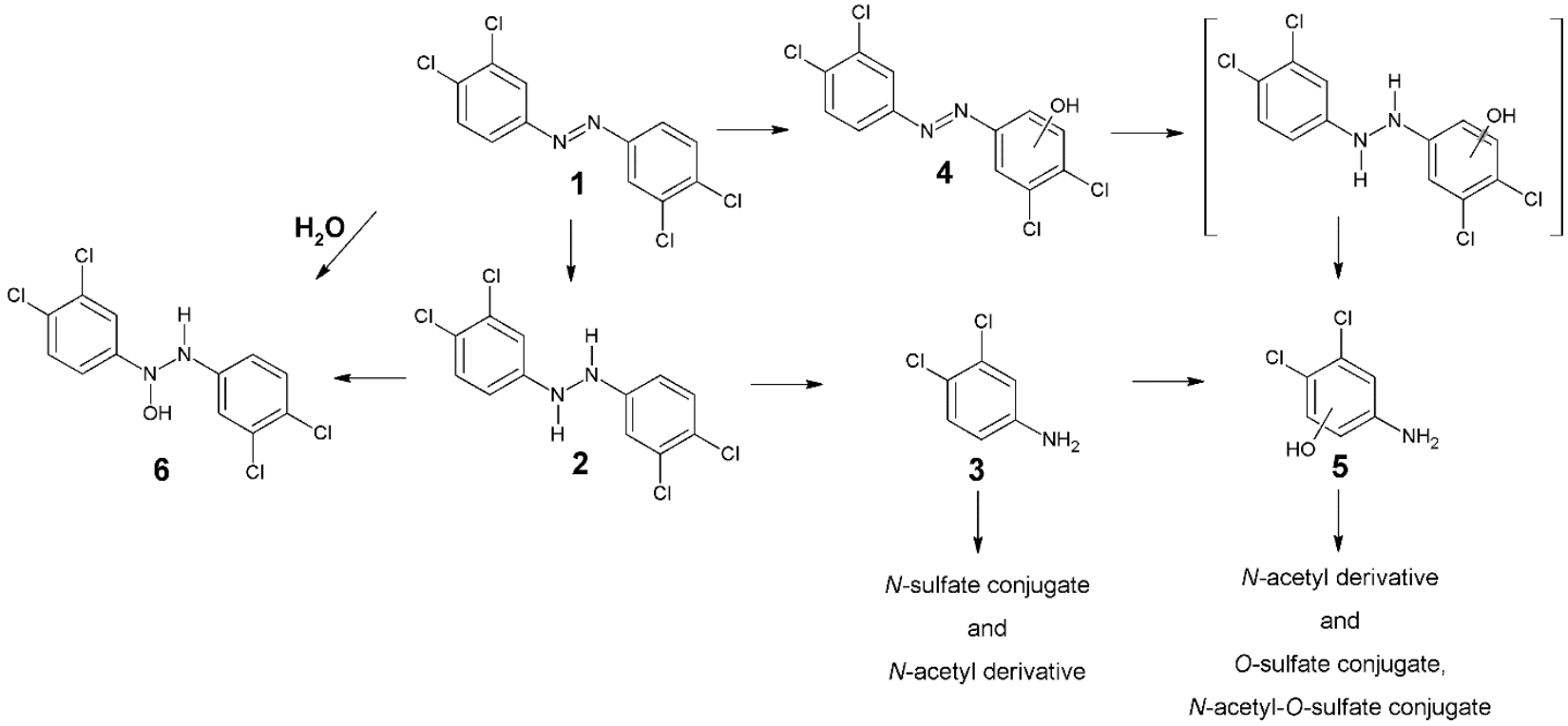
Collectively, the concentrations of TCAB in rat tissues increased in a dose-dependent manner regardless of route of administration. After parenteral administration, TCAB was distributed rapidly into tissues ($t_{1/2\alpha}$, 1 hour), mainly adipose tissue. Terminal elimination of TCAB from the blood was also fairly rapid ($t_{1/2\beta}$, 4–6 hours), whereas elimination of TCAB from adipose tissue was slower (Pillai et al., 1996; NTP, 2010).

4.1.4 Metabolism and modulation of metabolic enzymes

(a) Humans

No data were available to the Working Group.

Fig. 4.1 Biotransformation of 3,3′,4,4′-tetrachloroazobenzene (TCAB) in the rat



(1) 3,3′,4,4′-tetrachloroazobenzene (TCAB); (2) 3,3′,4,4′-tetrachlorohydrazobenzene; (3) 3,4-dichloroaniline; (4) TCAB phenol; (5) hydroxylated 3,4-dichloroaniline; (6) *N*-hydroxy-3,3′,4,4′-tetrachlorohydrazobenzene. The structure in square brackets is a putative intermediate, because it has not been isolated. Putative dechlorination reactions are not shown. Adapted with permission from: 3,3′,4,4′-Tetrachloroazobenzene absorption, disposition, and metabolism in male Fischer 344 rats, Drug Metab Dispos, 24(2):238–244, ©ASPET (1996) (Pillai et al., 1996), with information from Hsia & Kreamer (1981).

(b) Experimental systems

The azo linkage in TCAB makes it highly susceptible to metabolic biotransformation. The metabolic pathway for TCAB given in Fig. 4.1 is based on data obtained in rats in vivo and in vitro (Hsia & Kreamer, 1981; Pillai et al., 1996). Extensive azo reduction of TCAB (1, Fig. 4.1) to 3,4-dichloroaniline metabolites (3, Fig. 4.1) via 3,3′,4,4′-tetrachlorohydrazobenzene (2, Fig. 4.1) after oral administration in rats, presumably by gut microbes, decreases the systemic absorption of TCAB (Pillai et al., 1996). [The Working Group noted that the dichloroaniline metabolites have structural alerts that suggest potential toxicity.] Azo reduction also probably occurs in rat liver, because rat liver microsomes could catalyse this reaction via cytochrome P450 monooxygenases (Hsia & Kreamer, 1981). In addition to reductive metabolism, TCAB can be oxidized by cytochrome P450s to a major metabolite named TCAB phenol (4, Fig. 4.1) (Hsia & Kreamer, 1981). It is likely that TCAB phenol undergoes further azo reduction, giving hydroxylated chloroaniline derivatives (5, Fig. 4.1). This is supported by the identification of several derivatives of dichloroaniline and their sulfate conjugates in the urine of rats given an oral dose of [^{14}C]-labelled TCAB, including an *O*-sulfate conjugate of ring-hydroxylated *N*-acetyl-3,4-dichloroaniline that accounted for about 25% of the total radiolabel in the urine (Pillai et al., 1996). [The Working Group noted that compared with the lipophilic TCAB, the dichloroaniline conjugates would be rapidly eliminated from the body.] Monochloroaniline derivatives were also detected in rat urine, indicating dechlorination (Pillai et al., 1996). In addition to the urinary metabolites, the main metabolite in rat bile was putatively identified as *N*-hydroxy-3,3′,4,4′-tetrachlorohydrazobenzene (6, Fig. 4.1); [the Working Group noted that this metabolite could be produced either by the hydration of the azo linkage in TCAB or by oxidation of the hydroazo linkage in 3,3′,4,4′-tetrachlorohydrazobenzene] (2; Fig. 4.1) (Hsia & Kreamer, 1981). During incubation of rat liver microsomes with [^{14}C]-labelled TCAB, a portion of the radiolabel (6% after 60 minutes) was irreversibly bound to the microsomal pellet, suggesting covalent modification of macromolecules by a TCAB-derived reactive metabolite (Hsia & Kreamer, 1981). Covalent binding was dependent on nicotinamide adenine dinucleotide phosphate reduced form (NADPH) and could be inhibited by monooxygenase inhibitors. The reactive metabolite was not identified (Hsia & Kreamer, 1981).

TCAB interacts with the AhR with a binding affinity (K_d) of 1.1 nM (Poland et al., 1976). TCAB was further shown to be a potent inducer of hepatic aryl hydrocarbon hydroxylase in chicken embryos (Poland et al., 1976). In male Sprague-Dawley rats, TCAB (25 mg/kg bw per day, for 5 days) increased liver-to-body weight ratios, and increased hepatic cytochrome P450 content (2.7-fold vs control animals) in a dose-dependent manner (Hsia & Kreamer, 1979a). Consequently, TCAB has been used as an experimental tool to induce hepatic cytochrome P450 activities in animals (Saint-Ruf et al., 1979; Keys et al., 1985; Shaddock et al., 1989; McMillan et al., 1990). Furthermore, the TCAB congener 3,3′,4,4′-tetrachloroazoxybenzene was also shown to be an effective inducer of hepatic monooxygenase activity (McMillan et al., 1990). Receptor-mediated effects involving the AhR pathway are further discussed in Section 4.2.1(a). No studies were found to indicate that TCAB is a ligand for the xenobiotic receptors pregnane X receptor (PXR) or constitutive androstane receptor (CAR).

4.1.5 Excretion

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

TCAB-derived metabolites are excreted in the faeces and urine (Burant & Hsia, 1984; Pillai et al., 1996). Male Sprague-Dawley rats treated with [^{14}C]-labelled TCAB (10 mg/rat, by gavage) had excreted 55% of the administered dose in the faeces and 27% in the urine over a 48-hour period (Burant & Hsia, 1984). Male Fischer rats treated with [^{14}C]-labelled TCAB (3.2 and 32 mg/kg bw, by gavage) also excreted significant amounts of radiolabel in the faeces (53–56% of the administered dose) and the urine (39–45% of the administered dose) over a 48-hour period (Pillai et al., 1996). In the urine, no parent TCAB residue was found, and the *O*-sulfate conjugate of ring-hydroxylated *N*-acetyl-3,4-dichloroaniline accounted for 25% of the radiolabel. Modest differences between rat strains were noted in urinary excretion; over a 24-hour period, Sprague-Dawley rats had excreted 20% (Burant & Hsia, 1984) and Fischer rats had excreted 30–40% (Pillai et al., 1996) of the administered dose.

Faecal elimination of [^{14}C]-labelled TCAB equivalents was mainly due to biliary excretion of a [^{14}C]-labelled TCAB metabolite into the gastrointestinal tract and its subsequent excretion in the faeces (Pillai et al., 1996). After intravenous administration of [^{14}C]-labelled TCAB (3.2 mg/kg bw), 33% of the administered dose was excreted in the bile within 6 hours, whereas only 21% was eliminated in the faeces by 24 hours. [The Working Group noted that the difference was due to enterohepatic recirculation of [^{14}C]-labelled TCAB equivalents.] The main biliary metabolite was putatively identified as *N*-hydroxy-3,3′,4,4′-tetrachlorohydrazobenzene (6, Fig. 4.1; Pillai et al., 1996). No unchanged TCAB was detected in faecal extracts after intravenous administration of [^{14}C]-labelled TCAB; the fraction of faecal radiolabel attributable to unchanged (and putatively unabsorbed) [^{14}C]-labelled TCAB after an oral dose was not determined (Pillai et al. 1996).

Together the data indicated that TCAB metabolites are excreted readily and that both urine and faeces are important routes of excretion.

4.2 Mechanisms of carcinogenesis

This section summarizes in the following order the available evidence for the key characteristics of carcinogens (Smith et al., 2016), concerning whether TCAB modulates receptor-mediated effects; induces chronic inflammation; alters cell proliferation, cell death, or nutrient supply; and is genotoxic. For the other key characteristics of carcinogens, insufficient data were available for evaluation.

4.2.1 Receptor-mediated effects

(a) Aryl hydrogen receptor pathway

(i) Humans

There is no direct evidence that TCAB binds to the human AhR; however, several studies were available on chloracne (a skin condition characterized by comedones and retention cysts), which is pathognomonic for AhR activation in humans (Poland et al., 1976). Several series of chloracne cases have been reported among workers at plants where dichloroaniline herbicides were produced (Taylor et al., 1977; Morse et al., 1979; Scarisbrick & Martin, 1981; McDonagh et al., 1993). One plant produced methazole, one produced propanil and carbamate pesticides, two others produced dichloroaniline and diuron, and another plant was described only as manufacturing dichloroaniline derivatives. In addition to the end products, TCAB and 3,4,3′,4′-tetrachloroazoxybenzene (TCAOB) were reported as contaminants and other chemicals used in production were present. Workers were apparently exposed to chemicals as a result of an accident in one plant and through poor housekeeping practices in others. However, neither individual exposure data for the chloracne cases nor quantitative data

on the environmental levels of TCAB or any other agent in the plants were reported. [Consequently, the Working Group noted that although development of chloracne was associated with exposure to TCAB, the possibility that other chemicals were involved could not be ruled out.]

No other data from humans were available to the Working Group, including on human AhRs, or concerning AhR activation in human cells in vitro.

(ii) Non-human mammalian experimental systems in vivo

Poland et al. (1976) reported that TCAB and TCAOB induced hepatic aryl hydrocarbon hydroxylase activity, a marker of CYP1A1 activity and AhR activation, in male C57/Bl6 mice. TCAB and TCAOB were, respectively, approximately 20 and 8000 times less potent than TCDD.

Several studies in rodents examined non-neoplastic effects that have been associated with activation of the AhR. In $B6C3F_1$ mice exposed by oral gavage for 13 weeks, TCAB increased liver weights and thymic atrophy (NTP, 1998, 2010; van Birgelen et al., 1999). Chronic non-neoplastic effects of TCAB in female Sprague-Dawley rats included hyperplastic and proliferative lesions in the liver, thyroid gland, forestomach, oral mucosa, and adrenal cortex (NTP, 2010), similar to those observed with AhR agonist chemicals (NTP, 2006a, b, c).

In male Sprague-Dawley rats fed diets containing TCAB for up to 120 days, decreased body-weight gains, increased liver and spleen weights, and decreased testis weights were reported (Hsia et al., 1980, 1982). The increased liver weights were accompanied by increases in hepatic cytochrome P448 and aryl hydrocarbon hydroxylase activity (Hsia et al., 1980). In Sprague-Dawley and Fisher 344/N rats exposed by oral gavage for 13 weeks, TCAB induced hepatic CYP1A1 and CYP1A2, in association with increased liver weights and thymic atrophy (NTP, 1998). Weanling male Sprague-Dawley rats given TCAB as two weekly intraperitoneal doses (25 mg/kg bw) for up to 28 days developed a wasting syndrome and thymic atrophy (Hsia & Kreamer, 1985). Thymic atrophy, increased liver weights, depressed levels of hepatic gluconeogenic enzymes, and increased levels of total hepatic cytochrome P450 were also seen in male Sprague-Dawley rats given intraperitoneal injections of TCAB twice per week for 7 and 28 days (Hsia et al., 1982; Hsia & Kreamer, 1985). In immature male Wistar rats, TCAB (300 µg/kg bw, intraperitoneal) and other halogenated hydrocarbons induced hepatic testosterone 7α-hydroxylase, inhibited other testosterone hydroxylases, and decreased androstenedione formation (Keys et al., 1985). These effects on testosterone metabolism were correlated with decreased body weight.

In a study of long-term toxicity and carcinogenicity in female Sprague-Dawley rats, TCAB induced cystic keratinizing epithelioma of the lung, cholangiocarcinoma of the liver, and gingival squamous cell carcinoma of the oral mucosa (NTP, 2010). These effects were observed in similar studies with TCDD, 2,3,4,6,7-pentachlorodibenzofuran, and 3,3′,4,4′,5-pentachlorobiphenyl (PCB-126), all of which are AhR agonists (NTP, 2006a, b, c, 2010).

[The Working Group noted that multiple studies in mice and rats have reported effects that are hallmarks of, or consistent with, AhR activation.]

As noted below, there were several neoplastic and non-neoplastic findings with TCAB that were not observed in any of the bioassays with AhR agonist chemicals (NTP, 2006a, b, c, 2010).

(iii) Non-human mammalian experimental systems in vitro

Poland et al. (1976) first reported that TCAB and TCAOB bound the murine AhR from C57BL/6J mouse liver cytosol with an equilibrium dissociation constant about one fifth that of TCDD.

Xiao et al. (2016) evaluated the ability of TCAB to induce ethoxyresorufin-*O*-deethylase (EROD) activity, a marker for CYP1A1 and AhR activation, in a rat hepatoma cell line (H4IIE cells). TCAB induced EROD activity, like TCDD did, but was ~1.2×10^{-5} times as potent.

(iv) Non-mammalian experimental systems

Poland et al. (1976) reported an increased incidence of aryl hydrocarbon hydroxylase activity, a marker for CYP1A1 and AhR activation, in chicken embryos exposed to TCAB. In a rainbow trout liver cell line (RTL-W1 cells), TCAB induced EROD activity, like TCDD did, but was ~8.7×10^{-4} times as potent (Xiao et al., 2016). In a test for toxicity in the zebrafish embryo, TCAB induced a variety of cardiovascular disorders including heart oedema and heart malformations as well as yolk malformations, which have also been observed with AhR agonists (Xiao et al., 2016).

(b) Other receptors

TCAB (1000 µg/L) produced responses of less than 10% of maximum in the estrogen receptor (ER) and androgen receptor (AR)-CALUX assays (reporter cell lines derived from human osteosarcoma U2OS cells) (Xiao et al., 2016).

TCAB decreased circulating thyroxine concentrations, but had no effect on triiodothyronine and thyroid stimulating hormones, in Fischer 344/N (NTP, 1998) and Sprague-Dawley rats (NTP, 2010) after 13 weeks of exposure. These effects on thyroid hormones are similar to those reported for TCDD (NTP, 2006a). Decreased thyroxine concentrations were seen in male offspring in the NTP evaluation of developmental neurotoxicity of TCAB in Sprague-Dawley rats (dams exposed before mating, and male offspring exposed on postnatal days 4–21) (Harry et al., 2014). The decreased thyroxine concentrations were associated with histopathological changes in the hippocampus, suggesting that the decreases in circulating hormones resulted in developmental neurotoxicity (Harry et al., 2014).

4.2.2 Inflammation and immunosuppression

(a) Humans

As noted above (see Section 4.2.1), several case series of chloracne have been reported among workers at plants where dichloroaniline herbicides were produced and where TCAB was one of the exposures. Chloracne is an inflammatory process that leads to keratinous plugs in the skin pores resulting in cysts and dark pustules.

No other data from humans were available to the Working Group.

(b) Experimental systems

TCAB induced acnegenic effects in the rabbit ear bioassay (Hill et al., 1981). Solutions (0.1 mL) containing TCAB were painted onto the left ear of rabbits daily for 5 days (the right ear was used as the untreated control). Chloracne-like lesions were observed in $B6C3F_1$ mice in a study of long-term toxicity and carcinogenicity by the NTP (2010). The findings consisted of gross inflammatory skin lesions, characterized histologically by inflammation, fibrosis, hyperplasia, and ulcers (NTP, 2010). Chronic active inflammation of the ureter (males) and the lung (females) were also observed in mice exposed to TCAB for 2 years (Ramot et al., 2009; NTP, 2010). Inflammation of the thyroid, blood vessels, pancreas, and nose were observed in rats (Ramot et al., 2009; NTP, 2010). Neoplastic effects occurred in many of the tissues in which inflammation or chronic active inflammation was also observed.

4.2.3 Altered cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In Fisher 344/N (NTP, 1998) and Sprague-Dawley (NTP, 2010) rats, and in B6C3F$_1$ mice (NTP, 1998) exposed for 13 weeks, TCAB induced hyperplasia of the forestomach in males and females. In male and female rats, TCAB increased the incidence of oral gingival hyperplasia and of hyperplasia of the zona fasciculata of the adrenal cortex (NTP, 2010). Hyperplasia of the follicular cells in the thyroid gland was seen in male rats (NTP, 1998; NTP, 2010). An increased incidence of haematopoietic cell proliferation in the spleen was observed in TCAB-exposed male and female rats and mice (NTP, 1998, 2010; van Birgelen et al., 1999). In male and female mice, epidermal hyperplasia as well as glandular stomach focal epithelial hyperplasia and urinary bladder transitional cell hyperplasia were observed (NTP, 2010). Neoplastic effects occurred in many of the tissues in which hyperplasia was also observed.

With bromodeoxyuridine labelling, no alterations in cell proliferation were observed in the liver of Sprague-Dawley rats treated with TCAB for 13 weeks (NTP, 2010). Ramot et al. (2012) found a dose-related increase in the incidence of gingival squamous cell hyperplasia and of gingival cystic keratinizing hyperplasia in all treated Sprague-Dawley rats (but not of cystic keratinizing hyperplasia in males at the highest dose), using proliferating cell nuclear antigen staining as a marker of proliferation.

4.2.4 Genetic and related effects

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.1.

Bhusari et al. (2014) evaluated a subset of the tumours reported by NTP (2010) (see Section 3) for alterations in *Kras* and *Tp53*, two genes involved in human cancers. Urethral tumours from male and female mice had transforming point mutations in *Kras* (38%) and *Tp53* (63%). Similar rates of these mutations were observed in the mouse pulmonary carcinomas (*Kras*, 36%; *Tp53*, 55%). The mutations were not observed in the two pulmonary tumours that occurred in untreated animals. [The Working Group noted that a small subset of the tumours was available for analysis, and only two pulmonary carcinomas and no urethral tumours from control animals were examined. In addition, spontaneous or chemically induced transitional cell carcinomas of the urethra or ureter of B6C3F$_1$ mice were not reported in any other 2-year NTP cancer bioassays (approximately 600 studies were available). The increase in frequency of point mutations in *Kras* and *Tp53* suggested that TCAB or its metabolites may target guanine or cytosine bases.]

In B6C3F$_1$ mice, TCAB gave negative results in a test for micronucleus formation in the bone marrow in male mice after 3 days of intraperitoneal exposure at doses as high as 200 mg/kg bw per day (Witt et al., 2000). Increases in the frequency of micronucleated normochromatic erythrocytes were observed in male mice after 13 weeks of exposure at 10 and 30 mg/kg bw per day (NTP, 1998; Witt et al., 2000). In female mice exposed to TCAB for 13 weeks, there was a significant increasing trend in the frequency of micronucleated normochromatic erythrocytes, but results for the individual dose levels were not statistically significantly different from those of controls (NTP, 1998; Witt et al., 2000).

(ii) Non-human mammalian cells in vitro

See Table 4.2.

In rat primary hepatocytes isolated from untreated male Sprague-Dawley rats, overnight treatment with TCAB did not significantly increase unscheduled DNA synthesis (McMillan et al., 1988). TCAB also did not induce unscheduled DNA synthesis in hepatocytes isolated from naive rats in a separate study (Shaddock et al.,

Table 4.1 Genetic and related effects of 3,3′,4,4′-tetrachloroazobenzene (TCAB) in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Reference
Tp53 and *Kras* mutation	Mouse, B6C3F$_1$ (M, F)	Urethral (M) and pulmonary carcinomas (M, F)	+	10 mg/kg bw	i.g., 2 yr, 3, 10, or 30 mg/kg bw, 5 d/wk	Bhusari et al. (2014)
Micronucleus formation	Mouse, B6C3F$_1$ (M)	Bone marrow (PCE)	–	200 mg/kg	i.p., 3×, 50, 100, 150, or 200 mg/kg	NTP (1998); Witt et al. (2000)
	Mouse, B6C3F$_1$ (M, F)	Peripheral blood erythrocytes	+	10 mg/kg	i.g., 13 wk, 0.1, 1, 3, 10, or 30 mg/kg, 5 d/wk	

[a] +, positive; –, negative; the level of significance was set at $P < 0.05$ in all cases

bw, body weight; d, day; F, female; HID, highest ineffective dose; i.g. intragastric; i.p., intraperitoneal; LED, lowest effective dose; M, male; PCE, polychromatic erythrocytes; wk, week(s)

Table 4.2 Genetic and related effects of 3,3′,4,4′-tetrachloroazobenzene (TCAB) in non-human mammalian cells in vitro

End-point	Species, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Unscheduled DNA synthesis	Rat, Sprague-Dawley, hepatocytes	–	–	6.4 µg/mL		McMillan et al. (1988)
Unscheduled DNA synthesis	Rat, Sprague-Dawley, hepatocytes	–	+	3.2 µg/mL		Shaddock et al. (1989)
Unscheduled DNA synthesis	Rat, Sprague-Dawley, hepatocytes	(+)	NT	10 µM	Short (3 h) incubation, unclear whether triplicates were from the same or different samples	Hsia & Kreamer (1979b)
Hgprt mutation	Chinese hamster, CHO-K1 ovary cell line	–	–	14.4 µg/mL		McMillan et al. (1988)

[a] +, positive; –, negative; (+), positive in a study with limited quality; the level of significance was set at $P < 0.05$ in all cases

h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; Hgprt, hypoxanthine guanine phosphoribosyl transferase

Table 4.3 Genetic and related effects of 3,3′,4,4′-tetrachloroazobenzene (TCAB) in bacteria (*Salmonella typhimurium*)

Strain	End-point	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
TA97	Reverse mutation	–	+	50 µg/plate	NTP (1998)
TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	10 000 µg/plate	NTP (1998)
TA97, TA98, TA100, TA104	Reverse mutation	–	–	250 µg/plate	McMillan et al. (1988)
TA98, TA100	Reverse mutation	+	+	100 µg/plate	Gilbert et al. (1980)

[a] +, positive; –, negative; the level of significance was set at $P < 0.05$ in all cases
HIC, highest ineffective concentration; LEC, lowest effective concentration

1989). However, TCAB induced unscheduled DNA synthesis when the rats were pretreated with metabolic enzyme inducers, giving positive results at concentrations of 3.2 µg/mL (phenobarbital pretreated) or 6.4 µg/mL (Aroclor 1254 and TCAB pretreated) or higher (Shaddock et al., 1989). TCAB was not mutagenic in the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) assay in Chinese hamster ovary cells with or without metabolic activation (S9) (McMillan et al., 1988).

(iii) Non-mammalian systems

See Table 4.3.

TCAB gave positive results in *Salmonella typhimurium* strain TA97 in the presence of metabolic activation (S9), but not in strains TA98, TA100, TA1535, and TA1537 with or without metabolic activation (NTP, 1998). McMillan et al. (1988) reported negative results for TCAB in *S. typhimurium* strains TA97, TA98, TA100, and TA104 with or without metabolic activation. Gilbert et al. (1980) found that TCAB gave positive results in TA98 and TA100 with and without metabolic activation.

4.3 Data relevant to comparisons across agents and end-points

TCAB was not tested in high-throughput screening assays carried out by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast™) programmes of the government of the USA; for relevant results for other chemicals reviewed in the present volume, see Section 4.3 of the *Monograph* on pentachlorophenol in the present volume.

4.4 Cancer susceptibility data

No data were available to the Working Group.

4.5 Other adverse effects

4.5.1 Humans

With the exception of chloracne, described above, no data were available to the Working Group.

4.5.2 Experimental systems

Long-term exposure to TCAB in rodents resulted in a broad range of adverse effects across many tissues (NTP, 2010). In addition to those noted above (see Section 4.2), atrophy

was observed in the lymph nodes, spleen, and pancreas in male and female rats, and in the clitoral gland, ovaries, thymus, and spleen in mice (NTP, 2010).

5. Summary of Data Reported

5.1 Exposure data

3,3′,4,4′-Tetrachloroazobenzene (TCAB) is not commercially manufactured, but is formed as an unwanted by-product in the manufacture of 3,4-dichloroaniline and its herbicidal derivatives. TCAB has been measured at concentrations up to 1400 μg/g in propanil, and at up to 28 μg/g in linuron, diuron, or neburon formulations. Environmental contamination by TCAB occurs from the degradation of chloroanilide herbicides in the soil by peroxide-producing microorganisms, and by the photolysis and biolysis of 3,4-dichloroaniline. The use of propanil and other chloroanilide herbicides has increased substantially over the past two decades; current annual use in the USA is estimated to exceed 6 million pounds [~2700 tonnes]. However, no measurements of TCAB exposure in occupational settings or in the general population were reported. Occupational exposure may include workers involved in the manufacture of aniline herbicides, applicators who spray or mix aniline herbicide-containing formulations, and farm workers who enter fields after spraying. The general population may be exposed to TCAB from residues on food, or from living near areas where aniline herbicides are applied. TCAB sorbs strongly to soils and has been detected in the top 10 cm of soil up to 2 years after application of propanil.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

TCAB was tested for carcinogenicity in one gavage study in mice and one gavage study in rats.

In male mice, there was an increase in the incidence of transitional cell carcinoma of the urethra, bronchioloalveolar adenoma of the lung, and squamous cell carcinoma of the forestomach.

In female mice, there was an increase in the incidence of fibrosarcoma of the skin, malignant schwannoma or fibrosarcoma (combined) of the skin, bronchioloalveolar carcinoma of the lung, bronchioloalveolar adenoma or carcinoma (combined) of the lung, squamous cell carcinoma of the forestomach, and malignant lymphoma. There were also two instances of the rare tumour transitional cell carcinoma of the urethra in females at the highest dose.

In male rats, there was an increase in the incidence of cystic keratinizing epithelioma of the lung, cholangiocarcinoma of the liver, gingival squamous cell carcinoma of the oral mucosa, and follicular cell adenoma of the thyroid gland, and a positive trend in the incidence of malignant schwannoma.

In female rats, there was an increase in the incidence of cystic keratinizing epithelioma of the lung, gingival squamous cell carcinoma of the oral mucosa, and squamous cell papilloma or carcinoma (combined) of the forestomach. Rare cholangiocarcinomas of the liver were reported in treated females.

5.4 Mechanistic and other relevant data

No data were available on the absorption of TCAB in humans after oral, dermal, or inhalation exposures. The bioavailability of a bolus oral dose of TCAB given to rats is ~30% of the administered dose. Adipose tissue is a main storage depot after distribution of TCAB. TCAB

is rapidly metabolized, with extensive azo reduction in the gut and liver to give 3,4-dichloroaniline metabolites. TCAB metabolites are excreted readily in the urine and faeces.

With respect to the key characteristics of carcinogens, adequate data were available to evaluate whether TCAB modulates receptor-mediated effects; induces chronic inflammation; alters cell proliferation, cell death, or nutrient supply; and is genotoxic.

There is *strong* evidence that TCAB modulates receptor-mediated effects, but data in exposed humans and human cells are sparse. Chloracne has been reported in four case series of workers involved in the production of dichloroaniline herbicides, with exposures to TCAB, 3,4,3′,4′-tetrachloroazoxybenzene (TCAOB), and other chemicals. Chloracne is pathognomonic for activation of the aryl hydrocarbon receptor (AhR) and has been observed in experimental studies of rabbits and mice treated with TCAB. TCAB activates the AhR in vivo in rats, mice, and chicken embryos. In long-term studies in rodents, exposure to TCAB induced cytochrome P450s (CYP1A1 and CYP1A2), caused wasting syndrome, increased liver weights, decreased circulating thyroxine concentrations, and induced thymic atrophy. These effects are consistent with or are hallmarks of AhR activation, and are observed after AhR agonist exposures. TCAB activates the AhR in vitro in mice, rats, and rainbow trout.

There is *strong* evidence that TCAB induces chronic inflammation, but data in exposed humans and human cells are sparse. Chloracne, which is in part an inflammatory response, has been observed in the dichloroaniline-herbicide production workers mentioned previously, as well as in experimental studies of rabbits and mice treated with TCAB. Chronic inflammation was observed in numerous tissue types in rats and mice exposed to TCAB for up to 2 years. These inflammatory responses are consistent with those induced by AhR agonists in long-term studies.

There is *strong* evidence that TCAB alters cell proliferation, cell death, or nutrient supply. In experimental animals, long-term exposure to TCAB induces hyperplasia in numerous tissue types.

There is *weak* evidence that TCAB is genotoxic. In mice, 13 weeks of dietary exposure to TCAB induced increases in the frequency of micronucleus formation in male and female mice. However, short-term exposure to TCAB in male mice did not alter the frequency of micronucleus formation. There are conflicting findings for genotoxicity in assays for bacterial mutagenesis with TCAB.

No evidence was available concerning cancer susceptibility.

Long-term exposure to TCAB resulted in a broad range of non-neoplastic adverse effects across many tissues in mice and rats.

In sum, TCAB activates the AhR in experimental systems in vitro and in vivo. TCAB displays a wide variety of effects that are also induced by AhR agonists, including pathognomonic effects such as chloracne.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 3,3′,4,4′-tetrachloroazobenzene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 3,3′,4,4′-tetrachloroazobenzene.

6.3 Overall evaluation

3,3′,4,4′-Tetrachloroazobenzene is *probably carcinogenic to humans* (*Group 2A*).

6.4 Rationale

3,3′,4,4′-Tetrachloroazobenzene is *probably carcinogenic to humans (Group 2A)* on the basis of its belonging to the class of agents that activate the aryl hydrocarbon receptor (AhR), including dioxins, polychlorinated biphenyls, and polybrominated biphenyls, that are categorized as Group 1 or Group 2A carcinogens. The rationale for this evaluation is as follows:

- In vitro, 3,3′,4,4′-tetrachloroazobenzene binds to the mouse AhR, and activates rat and rainbow trout AhRs.
- 3,3′,4,4′-Tetrachloroazobenzene induces a spectrum of tumours in rats and mice that includes those observed with other AhR agonists that are categorized in Group 1, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, polychlorinated biphenyl 126 (PCB-126), and 2,3,4,7,8-pentachlorodibenzofuran.
- 3,3′,4,4′-Tetrachloroazobenzene induces multiple non-neoplastic effects in mice, rats, rabbits, chickens, and zebrafish consistent with AhR activation, including chloracne (a response pathognomonic for AhR-mediated toxicity) in mice and rabbits.

References

Allinson G, Morita M (1995a). Bioaccumulation and toxic effects of elevated levels of 3,3′,4,4′-tetrachloroazobenzene (3,3′,4,4′-TCAB) towards aquatic organisms. II: Bioaccumulation and toxic effects of dietary 3,3′,4,4′-TCAB on the Japanese Medaka (*Oryzias latipes*). *Chemosphere*, 30(2):223–32. doi:10.1016/0045-6535(94)00385-8 PMID:7874471

Allinson G, Morita M (1995b). Bioaccumulation and toxic effects of elevated levels of 3,3′,4,4′-tetrachloroazobenzene (3,3′,4,4′-TCAB) towards aquatic organisms. III: Bioaccumulation and toxic effects of detrital 33′44′-TCAB on the aquatic snail, Indohiramakigai (*Indoplanorbis exustus*). *Chemosphere*, 30(2):233–42. doi:10.1016/0045-6535(94)00386-9 PMID:7874472

Bartha R, Linke HA, Pramer D (1968). Pesticide transformations: production of chloroazobenzenes from chloroanilines. *Science*, 161(3841):582–3. doi:10.1126/science.161.3841.582 PMID:5663300

Bartha R, Pramer D (1969). Transformation of the herbicide methyl-N-(3,4-dichlorophenyl)-carbamate (Swep) in soil. *Bull Environ Contam Toxicol*, 4(4):240–5. doi:10.1007/BF01557982 PMID:24185499

Bhusari S, Malarkey DE, Hong HH, Wang Y, Masinde T, Nolan M, et al. (2014). Mutation spectra of Kras and Tp53 in urethral and lung neoplasms in B6C3F1 mice treated with 3,3′,4,4′-tetrachloroazobenzene. *Toxicol Pathol*, 42(3):555–64. doi:10.1177/0192623313491169 PMID:23703846

Bunce NJ, Corke CT, Merrick RL, Bright JH (1979). 3,3′,4,4′-Tetrachloroazobenzene as a contaminant in commercial propanil. *Chemosphere*, 8(5):283–4. doi:10.1016/0045-6535(79)90110-3

Burant CF, Hsia MT (1984). Excretion and distribution of two occupational toxicants, tetrachloroazobenzene and tetrachloroazoxybenzene in the rat. *Toxicology*, 29(3):243–50. doi:10.1016/0300-483X(84)90025-8 PMID:6695385

Call DJ, Brooke LT, Kent RJ, Knuth ML, Anderson C, Moriarity C (1983). Toxicity, bioconcentration, and metabolism of the herbicide propanil (3′,4′-dichloropropionanilide) in freshwater fish. *Arch Environ Contam Toxicol*, 12(2):175–82. doi:10.1007/BF01059578 PMID:6847248

Carey AE, Yang HS, Wiersma GB, Tai H, Maxey RA, Dupuy AE Jr (1980). Residual concentrations of propanil, TCAB, and other pesticides in rice-growing of soils in the United States, 1972. *Pestic Monit J*, 14(1):23–5. PMID:7422467

Di Muccio A, Camoni I, Dommarco R (1984). 3,3′,4,4′-Tetrachloroazobenzene and 3,3′,4,4′-tetrachloroazoxybenzene in technical grade herbicides: propanil, diuron, linuron, and neburon. *Ecotoxicol Environ*

Saf, 8(6):511–5. doi:10.1016/0147-6513(84)90010-1 PMID:6510324

EPA (1985). Health and Environmental Effects Profile for TCAB, TCAOB, and TCHB. Cincinnati (OH), USA: United States Environmental Protection Agency.

EPA (2011). Pesticides industry sales and usage. 2006 and 2007 market estimates. Cincinnati (OH), USA: United States Environmental Protection Agency.

EPA OTS (1983). Skin absorption subacute tests with dichloroaniline and related materials. Cincinnati (OH), USA: United States Environmental Protection Agency, Office of Toxic Substances.

Gilbert P, Saint-Ruf G, Poncelet F, Mercier M (1980). Genetic effects of chlorinated anilines and azobenzenes on *Salmonella typhimurium*. *Arch Environ Contam Toxicol*, 9(5):533–41. doi:10.1007/BF01056933 PMID:7002063

Harry GJ, Hooth MJ, Vallant M, Behl M, Travlos GS, Howard JL, et al. (2014). Developmental neurotoxicity of 3,3′,4,4′-tetrachloroazobenzene with thyroxine deficit: Sensitivity of glia and dentate granule neurons in the absence of behavioral changes. *Toxics*, 2(3):496–532. doi:10.3390/toxics2030496 PMID:26029700

Hashimoto S, Schneider S, Yamamoto T, Morita M (1994). Aqueous solubility and octanol-water partition coefficient of 3,3′,4,4′-tetrachloroazobenzene. *Organohalogen Compd*, 20:125–8.

Hill RH Jr, Rollen ZJ, Kimbrough RD, Groce DF, Needham LL (1981). Tetrachloroazobenzene in 3,4-dichloroaniline and its herbicidal derivatives: propanil, diuron, linuron, and neburon. *Arch Environ Health*, 36(1):11–4. doi:10.1080/00039896.1981.10667599 PMID:6451202

Hsia MT, Burant CF, Kreamer BL, Schrankel KR (1982). Thymic atrophy induced by acute exposure of 3,3′,4,4′-tetrachloroazobenzene and 3,3′,4,4′-tetrachloroazoxybenzene in rats. *Toxicology*, 24(3–4):231–44. doi:10.1016/0300-483X(82)90005-1 PMID:6927643

Hsia MT, Kreamer BL (1979a). Induction of hepatic microsomal cytochrome P-448 by 3,3′,4,4′-tetrachloroazobenzene and the corresponding azoxy and hydrazo analogs. *Res Commun Chem Pathol Pharmacol*, 25(2):319–31. PMID:493716

Hsia MT, Kreamer BL (1979b). Induction of unscheduled DNA synthesis in suspensions of rat hepatocytes by an environmental toxicant, 3,3′4,4′-tetrachloroazobenzene. *Cancer Lett*, 6(4–5):207–12. doi:10.1016/S0304-3835(79)80035-X PMID:436117

Hsia MT, Kreamer BL (1981). Metabolism studies of 3,3′,4,4′-tetrachloroazobenzene. I. In vitro metabolic pathways with rat liver microsomes. *Chem Biol Interact*, 34(1):19–29. doi:10.1016/0009-2797(81)90086-7 PMID:7460075

Hsia MT, Kreamer BL (1985). Delayed wasting syndrome and alterations of liver gluconeogenic enzymes in rats exposed to the TCDD congener 3,3′,4,4′-tetrachloroazoxybenzene. *Toxicol Lett*, 25(3):247–58. doi:10.1016/0378-4274(85)90204-8 PMID:4012802

Hsia MT, Kreamer BL, Burant CF, Treutelaar MK (1980). General health effects of prolonged exposure to 3,3′,4,4′-tetrachloroazobenzene and 3,3′,4,4′-tetrachloroazoxybenzene in rats. *Drug Chem Toxicol*, 3(1):47–56. doi:10.3109/01480548009017832 PMID:7389582

Hughes AF, Corke CT (1974). Formation of tetrachloroazobenzene in some Canadian soils treated with propanil and 3,4-dichloroaniline. *Can J Microbiol*, 20(1):35–9. doi:10.1139/m74-006 PMID:4822779

Kearney PC, Smith RJ Jr, Plimmer JR, Guardia FS (1970). Propanil and TCAB residues in rice soils. *Weed Sci*, 18:464–6.

Keys B, Hlavinka M, Mason G, Safe S (1985). Modulation of rat hepatic microsomal testosterone hydroxylases by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related toxic isostereomers. *Can J Physiol Pharmacol*, 63(12):1537–42. doi:10.1139/y85-253 PMID:3830353

Lay MM, Ilnicki RD (1974). Peroxidase activity and propanil degradation in soil. *Weed Res*, 14(2):111–3. doi:10.1111/j.1365-3180.1974.tb01503.x

McDonagh AJ, Gawkrodger DJ, Walker AE (1993). Chloracne – study of an outbreak with new clinical observations. *Clin Exp Dermatol*, 18(6):523–5. doi:10.1111/j.1365-2230.1993.tb01021.x PMID:8252789

McMillan DC, Bradshaw TP, Hinson JA, Jollow DJ (1991). Role of metabolites in propanil-induced hemolytic anemia. *Toxicol Appl Pharmacol*, 110(1):70–8. doi:10.1016/0041-008X(91)90290-U PMID:1871773

McMillan DC, Leakey JE, Arlotto MP, McMillan JM, Hinson JA (1990). Metabolism of the arylamide herbicide propanil. II. Effects of propanil and its derivatives on hepatic microsomal drug-metabolizing enzymes in the rat. *Toxicol Appl Pharmacol*, 103(1):102–12. doi:10.1016/0041-008X(90)90266-W PMID:2315923

McMillan DC, Shaddock JG, Heflich RH, Casciano DA, Hinson JA (1988). Evaluation of propanil and its *N*-oxidized derivatives for genotoxicity in the *Salmonella typhimurium* reversion, Chinese hamster ovary/hypoxanthine guanine phosphoribosyl transferase, and rat hepatocyte/DNA repair assays. *Fundam Appl Toxicol*, 11(3):429–39. doi:10.1016/0272-0590(88)90107-8 PMID:3065118

Miller GC, Zisook R, Zepp R (1980). Photolysis of 3,4-dichloroaniline in natural waters. *J Agric Food Chem*, 28(6):1053–6. doi:10.1021/jf60232a010

Morse DL, Baker EL Jr, Kimbrough RD, Wisseman CL 3rd (1979). Propanil-chloracne and methomyl toxicity in workers of a pesticide manufacturing plant. *Clin Toxicol*, 15(1):13–21. doi:10.3109/15563657908992475 PMID:159154

NTP (1998). NTP technical report on the toxicity studies of 3,3′,4,4′-tetrachloroazobenzene (CAS No. 14047-09-7) administered by gavage to F344/N rats and B6C3F1 mice. *Toxic Rep Ser*, 65:1–F6. PMID:11986682

NTP (2006a). NTP technical report on the toxicology and carcinogenesis studies of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (CAS No. 1746-01-6) in female Harlan Sprague-Dawley rats (gavage studies). *Natl Toxicol Program Tech Rep Ser*, (521):4–232. PMID:16835633

NTP (2006b). NTP toxicology and carcinogenesis studies of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) in female Harlan Sprague-Dawley rats (gavage studies). *Natl Toxicol Program Tech Rep Ser*, (520):4–246. PMID:16628245

NTP (2006c). Toxicology and carcinogenesis studies of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (CAS No. 57117-31-4) in female Harlan Sprague-Dawley rats (gavage studies). *Natl Toxicol Program Tech Rep Ser*, (525):1–198. PMID:17160103

NTP (2010). Toxicology and carcinogenesis studies of 3,3′,4,4′-tetrachloroazobenzene (TCAB) (CAS No. 14047-09-7) in Harlan Sprague-Dawley rats and B6C3F1 mice (gavage studies). *Natl Toxicol Program Tech Rep Ser*, 558:1–206.

OEHHA (2012). Chemicals known to the state to cause cancer or reproductive toxicity. Proposition 65 list. California, USA: Office of Environmental Health Hazard Assessment. Available from: http://oehha.ca.gov/media/downloads/crnr/p65single072012.pdf.

OEHHA (2016). Preliminary screening information on possible pesticide classes for future consideration. Organophosphorus pesticides, neonicotinoid pesticides, anilide pesticides. Materials for July 28, 2016 Meeting of Scientific Guidance Panel for Biomonitoring California at the August 2013 meeting of the Scientific Guidance Panel (SGP). California, USA: Office of Environmental Health Hazard Assessment. Available from: http://www.biomonitoring.ca.gov/sites/default/files/downloads/PossiblePesticideClasses_072816.pdf.

Pillai UA, Ziegler TL, Wang DX, Kattnig MJ, McClure T, Liebler DC, et al. (1996). 3,3′,4,4′-Tetrachloroazobenzene absorption, disposition, and metabolism in male Fischer 344 rats. *Drug Metab Dispos*, 24(2):238–44. PMID:8742237

Poland A, Clover E, Kende AS, DeCamp M, Giandomenico CM (1976). 3,4,3′,4′-Tetrachloro azoxybenzene and azobenzene: potent inducers of aryl hydrocarbon hydroxylase. *Science*, 194(4265):627–30. doi:10.1126/science.136041 PMID:136041

Ramot Y, Nyska A, Lieuallen W, Maly A, Flake G, Kissling GE, et al. (2009). Inflammatory and chloracne-like skin lesions in B6C3F$_1$ mice exposed to 3,3′,4,4′-tetrachloroazobenzene for 2 years. *Toxicology*, 265(1–2):1–9. doi:10.1016/j.tox.2009.08.017 PMID:19737593

Ramot Y, Vered M, Malarkey DE, Hooth MJ, Painter JT, Dayan D, et al. (2012). Immunohistochemical features of 3,3′,4,4′-tetrachloroazobenzene-induced rat gingival lesions. *Toxicol Pathol*, 40(4):577–92. doi:10.1177/0192623311436185 PMID:22317924

Saint-Ruf G, Hien DP, Mercier M (1979). Effects of 3,3′,4,4′-tetrachloroazobenzene and 3,3′,4,4′-tetrachloroazoxybenzene on rat liver microsomes. *Bull Environ Contam Toxicol*, 22(1):638–42. doi:10.1007/BF02027000 PMID:486765

Scarisbrick DA, Martin JV (1981). Biochemical changes associated with chloracne in workers exposed to tetrachlorazobenzene and tetrachlorazoxybenzene. *J Soc Occup Med*, 31(4):158–63. doi:10.1093/occmed/31.4.158 PMID:6460133

Shaddock JG, Heflich RH, McMillan DC, Hinson JA, Casciano DA (1989). Pretreatment with mixed-function oxidase inducers increases the sensitivity of the hepatocyte/DNA repair assay. *Environ Mol Mutagen*, 13(4):281–8. doi:10.1002/em.2850130402 PMID:2737181

Singh J, Bingley R (1991). Levels of 3,3′,4,4′-tetrachloroazobenzene in propanil herbicide. *Bull Environ Contam Toxicol*, 47(6):822–6. doi:10.1007/BF01689510 PMID:1786453

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect*, 124(6):713–21. PMID:26600562

Still GG (1969). 3,4,3′,4′-Tetrachloroazobenzene: its translocation and metabolism in rice plants. *Weed Res.*, 9(3):211–7. doi:10.1111/j.1365-3180.1969.tb01476.x

Sundström G, Jansson B, Renberg L (1978). Determination of the toxic impurities 3,3′,4,4′-tetrachloroazobenzene and 3,3′,4,4′-tetrachloroazoxybenzene in commercial diuron, linuron and 3,4-dichloroaniline samples. *Chemosphere*, 7(12):973–9. doi:10.1016/0045-6535(78)90007-3

Taylor JS, Wuthrich RC, Lloyd KM, Poland A (1977). Chloracne from manufacture of a new herbicide. *Arch Dermatol*, 113(5):616–9. doi:10.1001/archderm.1977.01640050076010 PMID:140627

van Birgelen AP, Hébert CD, Wenk ML, Grimes LK, Chapin RE, Mahler J, et al. (1999). Toxicity of 3,3′,4,4′-tetrachloroazobenzene in rats and mice. *Toxicol Appl Pharmacol*, 156(2):147–59. doi:10.1006/taap.1999.8640 PMID:10198280

Witt KL, Zeiger E, Tice RR, van Birgelen AP (2000). The genetic toxicity of 3,3′,4,4′-tetrachloroazobenzene and 3,3′,4,4′-tetrachloroazoxybenzene: discordance between acute mouse bone marrow and subchronic mouse peripheral blood micronucleus test results. *Mutat Res*, 472(1–2):147–54. doi:10.1016/S1383-5718(00)00143-1 PMID:11113707

Worobey BL (1984). Fate of 3,3′,4,4′-tetrachloroazobenzene in soybean plants grown in treated soils. *Chemosphere*, 13(10):1103–11. doi:10.1016/0045-6535(84)90109-7

Worobey BL (1988). Translocation and disposition of [^{14}C] trans 3,4,3′,4′-tetrachloroazobenzene into carrots grown in treated soil. *Chemosphere*, 17(9):1727–34. doi:10.1016/0045-6535(88)90100-2

Xiao H, Kuckelkorn J, Nüßer LK, Floehr T, Hennig MP, Roß-Nickoll M, et al. (2016). The metabolite 3,4,3′,4′-tetrachloroazobenzene (TCAB) exerts a higher ecotoxicity than the parent compounds 3,4-dichloroaniline (3,4-DCA) and propanil. *Sci Total Environ*, 551–552:304–16. doi:10.1016/j.scitotenv.2016.02.019 PMID:26878642