Chem. Toxic Vol. 21, No. 6, pp. 825–832, 1983

0278-6915/83 $3.00 + 0.00

REVIEW OF RECENT TOXICOLOGY STUDIES ON *p*-DICHLOROBENZENE

1/1183

E. LOESER
Institute of Toxicology, Bayer AG, Wuppertal, Federal Republic of Germany

and

M. H. LITCHFIELD
Central Toxicology Laboratory, Imperial Chemical Industries plc, Macclesfield, Cheshire SK10 4TJ, England

(*Received* 11 *November* 1982; *revision received* 16 *March* 1983)

Abstract—Results from recent long-term inhalation, mutagenicity, embryotoxicity and metabolism studies on *p*-dichlorobenzene (*p*-DCB) are reviewed. Groups of male and female rats and female mice were exposed for 5 hr/day on 5 days/wk to *p*-DCB at concentrations of 0, 75 or 500 ppm for a total period of *c.* 76 wk (rats) or 57 wk (female mice) followed by 36 wk (rats) or 19 wk (female mice) without *p*-DCB exposure. No overt signs of toxicity were apparent at any exposure level nor were there treatment-related effects on the biochemical determinations, urine analyses or haematological parameters. Slightly elevated urinary coproporphyrin excretion and increased liver and kidney weights were regarded as treatment-related effects in the 500-ppm exposure group of the rats. The non-tumour and tumour pathology did not indicate any treatment-related effect in any group of either species. An embryotoxicity and teratology study on rats exposed to 0, 75, 200 or 500 ppm *p*-DCB vapour in air during the period of organogenesis did not demonstrate any signs of embryo- or foetotoxicity or teratogenicity at any exposure level. In a series of mutagenicity tests including the *Salmonella typhimurium*, dominant lethal and cytogenetic assays, *p*-DCB did not produce a mutagenic response. Studies using oral or inhalation routes of exposure demonstrated rapid metabolic transformation of *p*-DCB and excretion of the products, even after long-term exposure.

Introduction

p-Dichlorobenzene (1,4-dichlorobenzene; *p*-DCB) is a chlorinated aromatic compound which has been used for several decades in consumer products, in pest control and as an intermediate in chemical industries. These uses result in different exposure patterns for man, the three most important routes being vapour exposure, oral exposure via contaminated foods and local contact.

Despite the broad spectrum of use of *p*-DCB there are few reports of oral or inhalation toxicity of this compound, and no data are available on skin exposure. The basic data are derived from early publications by Zupko & Edwards (1949) and Hollingsworth, Rowe, Oyen *et al.* (1956). Various other studies have been published on metabolism, effects on the liver and mutagenicity, but there have been no studies on possible effects of long-term repeated exposure, on reproductive toxicity or on mutagenicity using test systems relevant for the estimation of risk. These data have been generated in the past few years and this review summarizes this unpublished work on long-term toxicity, embryotoxicity/teratogenicity and mutagenicity as well as some additional data on metabolism. Since inhalation is the predominant route of exposure most of these experiments involved treatment by inhalation. These experiments were also required to support the current TLV of 75 ppm set by the American Conference of Governmental Industrial Hygienists (1982).

Abbreviations: *p*-DCB = *p*-Dichlorobenzene; DMSO = dimethylsulphoxide; GC–ECD = gas chromatography with electron capture detector.

Use, physico-chemical properties and analytical determination of *p*-DCB

p-DCB is a white crystalline compound which is used as a repellent, space deodorant and fungicide (mildew-control agent) and as an intermediate in the dyestuff, deodorant, pesticide and pharmaceutical industries. It is produced by catalysed reactions of benzene with chlorine or with hydrogen chloride in the presence of air. These processes lead to mixtures of isomers from which *p*-DCB can be isolated by distillation, crystallization or extractive distillation.

Some important physico-chemical data for *p*-DCB are: mol wt 147.01; density (g/cm^3 at 55°C) 1.2675; m.p. + 53.08°C; b.p. 173.9°C; vapour pressure 13.3 mm Hg at 54.8°C; solubility (g/litre water) 0.069 at 20°C and 0.163 at 60°C; highly soluble in benzene, alcohols etc.

Several methods for the analytical determination of *p*-DCB and its metabolites in biological material are described in the literature (Langhorst & Nestrick, 1979; McKinney, Fishbein, Fletcher & Barthel, 1970; Schmidt, 1977). They differ in the pretreatment of the

samples for subsequent separation and determination by gas chromatography.

Long-term toxicity and carcinogenicity studies

Rat inhalation study

Groups of 76–79 rats of both sexes (SPF, Alderley Park Wistar-derived strain) were housed nine or ten per cage and exposed by inhalation to *p*-DCB vapour concentrations of 0 (air control), 75 or 500 ppm in air for 5 hr/day on 5 days/wk in stainless-steel chambers (2 m^3) for a total of 76 wk. The surviving rats were left unexposed for up to 36 wk following the exposure period.

p-DCB vapour atmospheres were generated by passing clean dry air through *p*-DCB (99.8%) in a water-jacketed vessel, which was thermostatically controlled at 55°C. The required exposure levels were obtained by adjusting the air flow through the *p*-DCB crystals into the exposure chamber. The exposure levels were monitored ever 2–3 days using an infra-red analyser. The overall deviation from the mean levels was less than 2%. Relative humidity and temperature were recorded throughout the study.

Clinical condition, body weights and food and water consumption were recorded at regular intervals. Blood biochemistry (blood urea, blood glucose and plasma alanine and aspartate-transaminase activities) and urine analyses were performed on five animals of each sex and group at wk 5, 14, 27, 40 and 52. Hepatic aminopyrine-demethylase activity was determined at wk 52–53 on five animals of each sex and group. Standard haematological data and urinary copoporphyrin excretion were recorded at wk 5, 14, 26–27, 40 and 52–53. Rats found dead, killed *in extremis* or scheduled for interim or terminal kills were subjected to detailed gross and histopathological examination. Some organ weights were recorded. The following tissues were examined histopathologically from all animals: adrenals, aorta, urinary bladder, caecum, colon, cervix, duodenum, epididymis, heart, ileum, lymph nodes, (cervical, thoracic and mesenteric), jejunum, kidneys, liver, lungs, mammary gland, oesophagus, ovaries, pancreas, prostate, salivary glands, seminal vesicle, spleen, stomach, testes, larynx, trachea, thymus, thyroid, uterus, voluntary muscle, Zymbal's gland, Harderian gland, bone (marrow), brain, sciatic nerve, nasal sinuses, pituitary, eye and spinal cord. Samples of fat, liver, plasma and urine from selected animals were analysed for metabolites of *p*-DCB at wk 26 and 76 and at the termination of the study (see section on metabolism, distribution and elimination).

No overt signs of exposure-related effect were reported and the mortality of the exposed rats was similar to that of the control animals throughout the study (Table 1). No treatment-related changes in body weight or food and water intake were seen. Although some changes in blood biochemistry and haematology parameters attained statistical significance, there was no evidence of a dose-related change. There was also no indication of an increased activity of hepatic aminopyrine demethylase.

Urinary protein and coproporphyrin output was slightly elevated in the 500-ppm group. This might have been related to functional changes in the liver or kidney, although there was no histological evidence for an effect in these organs. However, liver and kidney weights were increased, giving further evidence of an effect at 500 ppm. Small increases in the weights of heart and lung at 500 ppm were not related to any histological change and probably did not represent evidence of any effect of treatment. The changes in liver and kidney weights probably indicated milder manifestations of those changes seen at higher exposure levels by Hollingsworth *et al.* (1956). Apart from some suggestions of increased liver weight, no changes from control values could be discerned at the 75-ppm level and this dose was considered to be without toxicological effect (Riley, Chart, Doss *et al.* 1980a).

Table 1. Cumulative percentage mortality in long-term inhalation study of *p*-dichlorobenzene in rats

Exposure level (ppm)	Cumulative mortality* (%) at wk.		
	52	76	108
Males			
0	2.7	13.4	53.4
75	1.3	8.8	46.2
500	5.6	14.8	53.2
Females			
0	4.1	7.1	46.2
75	2.7	16.2	45.8
500	0.0	7.8	48.8

*In groups consisting originally of 76–79 rats.

No treatment-related effect on the incidence of tumours, their multiplicity or malignancy (Table 2) was seen in either the males or females exposed to *p*-DCB vapour at levels up to 500 ppm.

Mouse inhalation study

Groups of 75 female SPF Swiss mice (Alderley Park strain) were housed nine or ten per cage and exposed to *p*-DCB vapour concentrations of 0 (air control), 75 or 500 ppm in air for a total of 57 wk. The surviving mice were then placed in control air until the terminal kill at wk 75–76. (Originally, the study included similar groups of male mice. However, due to fighting among the males during the early part of the study, and also a probable occurrence of a respiratory infection, the male mice were terminated after wk 57, when *c.* 80% mortality had been approached.)

The exposure conditions in this study were the same as those used in the rat study described above. Clinical conditions were recorded at regular intervals. Mice found dead were subjected to gross pathology whenever possible. Detailed macropathology was performed on all mice killed *in extremis* or at terminal kill. Histopathology on the following tissues was performed on female mice that had received their treatment for at least 52 wk: adrenals, aorta, urinary bladder, caecum, colon, cervix, duodenum, heart, ileum, lymph nodes (cervical, thoracic and mesenteric), Harderian gland, jejunum, kidneys, liver, lungs, mammary gland, oesophagus, ovaries, pancreas, salivary glands, spleen, stomach, trachea, thymus, thyroid, uterus, voluntary muscle, Zymbal's gland, bone (marrow), brain, sciatic nerve, nasal sinuses, pituitary, eye and spinal cord.

The mortality in the female mice did not appear to be related to the exposure to *p*-DCB, the cumulative

Table 2 Summary of tumour incidence in long-term inhalation study of *p*-dichlorobenzene in rats

Organ diagnosis	Atmospheric concn (ppm) ...	No. of animals affected: Males 0	Males 75*	Males 500	Females 0	Females 75	Females 500
Buccal cavity—carcinoma†		3	1	4	0	1	1
Salivary glands—adenoma		0	1	0	0	0	1
—fibrosarcoma		0	1	1	0	1	0
—neurofibroma		1	0	0	0	0	0
—neurofibrosarcoma		0	1	0	0	0	0
—haemangioendothelialsarcoma		0	0	0	0	0	1
Stomach—papilloma		0	0	0	0	1	1
—keratoacanthoma		0	1	0	0	0	0
—adenocarcinoma		0	0	0	1	0	0
—fibrosarcoma		0	0	0	0	1	0
Intestines—fibroma		1	0	0	0	0	2
—fibrosarcoma		1	0	0	0	0	1
—leiomyoma		0	0	0	1	0	0
—adenocarcinoma		1	0	0	1	1	0
Liver—hepatocellular adenoma		0	0	0	1	0	0
Pancreas—acinar adenoma		0	1	1	0	0	0
Heart—neurofibroma		0	1	0	0	0	1
—neurofibrosarcoma		1	1	2	0	1	0
Brain—meningioma		7	0	4	6	3	1
—astrocytoma		1	2	1	1	0	0
—oligodendroglioma		1	0	0	0	0	0
—ependymoma		0	1	0	0	0	0
Adrenals—cortical adenoma		2	1	1	4	4	5
—cortical carcinoma		1	0	0	0	1	1
—phaeochromocytoma		3	4	2	3	3	0
Pancreas—islet-cell adenoma		0	1	0	0	1	0
—islet-cell carcinoma		3	1	3	1	0	2
Pituitary—adenoma		9	8	5	43	37	44
—carcinoma		2	0	0	3	3	0
—craniopharyngeal carcinoma		0	0	1	0	0	0
Parathyroid glands—adenoma		2	2	2	0	0	0
Thyroids—follicular adenoma		0	0	3	0	1	0
—parafollicular-cell adenoma		0	1	3	4	2	2
—parafollicular-cell carcinoma		0	0	0	0	1	2
Haemopoietic and lymphoreticular system							
—thymic lymphosarcoma		0	1	1	0	1	1
—focal lymphosarcoma		0	0	0	0	0	2
—generalized lymphosarcoma		0	0	1	1	0	0
—granulopoietic leukaemia		0	0	1	0	0	0
Mesenteric lymph node—fibrosarcoma		1	0	0	0	0	0
Thymus—lipoma		0	0	0	1	0	0
—thymoma		0	0	0	1	0	0
—carcinoma		0	1	0	0	0	0
Nasal passages—carcinoma		0	0	1	0	0	0
—fibrosarcoma		1	0	0	0	0	0
Skin—papilloma		1	1	0	0	1	1
—squamous-cell carcinoma		0	1	0	0	0	1
—basal-cell carcinoma		0	0	0	1	0	1
Subcutaneous tissues—lipoma		2	0	0	0	1	0
—fibroma		2	6	2	0	0	1
—fibrosarcoma		1	3	3	0	0	1
—histiocytoma		0	0	0	1	0	0
Kidneys—adenocarcinoma carcinoma		0	0	1	0	0	1
—mesenchymal tumour		0	0	0	1	0	0
—lipomatous tumour		0	0	0	1	0	0
Testes—interstitial-cell adenoma		3	3	3	—	—	—
—mesothelioma		1	1	5	—	—	—
Ovaries—granulosa thecal-cell tumour		—	—	—	1	4	4
—cystadenoma		—	—	—	0	1	0
—fibroleiomyoma leiomyoma		—	—	—	1	1	0
Uterus/cervix—polyp		—	—	—	8	8	0
—stromal-cell sarcoma		—	—	—	1	1	4
—leiomyoma		—	—	—	0	0	1
—haemangioendothelioma		—	—	—	0	1	0
—haemangioendothelial sarcoma		—	—	—	1	0	0
—squamous-cell carcinoma		—	—	—	0	1	0
—adenocarcinoma/carcinoma		—	—	—	0	4	0
Vagina—sarcoma		—	—	—	0	1	0
Mammary tissue—adenoma/fibroadenoma/fibroma		—	—	—	13	17	15
—adenocarcinoma/carcinoma		—	—	—	7	9	5
Zymbal's gland—squamous-cell carcinoma		1	1	0	0	0	1
Harderian gland—fibrosarcoma		0	0	1	0	0	0
Lachrymal gland—carcinoma		0	0	0	0	0	1
Retroperitoneal tissues—lipoma		1	0	0	0	0	0
Total no. of rats examined		60	60	60	61	61	58
No. of tumour-bearing rats		39	31	35	55	54	53
No. with multiple tumours		13	13	11	33	36	35
No. with malignant tumours		19	13	18	19	22	23

*Two animals from this group were excluded due to transposition of one animal from the 500-ppm group at the terminal kill

†Includes basal-cell and squamous carcinomas.

Table 3. Summary of tumour incidence in long-term inhalation study of *p*-dichlorobenzene in female mice

Organ/diagnosis	Atmospheric concn (ppm)...	No. of mice examined/affected 0	75	500
Adrenal	*No. examined...*	*47*	*44*	*50*
Phaeochromocytoma		0	0	1
Harderian gland	*No. examined...*	*35*	*37*	*34*
Adenoma		0	3	2
Pituitary	*No. examined...*	*39*	*39*	*45*
Adenoma		0	3	4
Liver	*No. examined...*	*48*	*47*	*50*
Type A nodule		0	1	1
Colon	*No. examined...*	*47*	*43*	*48*
Undifferentiated sarcoma		0	0	1
Lymphoreticular system	*No. examined...*	*48*	*47*	*50*
Generalized lymphosarcoma		7	8	1
Localized lymphosarcomas				
Caecum	*No. examined...*	*47*	*45*	*49*
Lymphosarcoma		0	1	0
Cervical lymph node	*No. examined...*	*46*	*46*	*46*
Lymphosarcoma		1	0	0
Lungs	*No. examined...*	*48*	*47*	*50*
Lymphosarcoma		1	0	0
Thymus	*No. examined...*	*39*	*40*	*44*
Lymphosarcoma		3	1	0
Spleen	*No. examined...*	*48*	*45*	*50*
Lymphosarcoma		0	0	2
Lungs	*No examined...*	*48*	*47*	*50*
Adenoma		3	3	2
Nasal sinus	*No. examined...*	*48*	*45*	*48*
Osteosarcoma		0	1	0
Mammary gland	*No. examined...*	*47*	*46*	*49*
Adenoma		0	1	0
Carcinoma		2	0	0
Ovary	*No. examined...*	*43*	*45*	*47*
Luteoma		1	0	0
Granulosa-theca cell		2	1	1
Fibroma		1	0	0
Uterus	*No examined...*	*47*	*46*	*50*
Leiomyoma		1	0	0
Fibroma		1	0	1
Adenoma		0	0	1
Undifferentiated sarcoma		0	1	0
Cervix	*No. examined...*	*44*	*41*	*47*
Leiomyoma		1	1	0
Leiomyosarcoma		1	0	1
Fibrosarcoma		1	0	0
Vascular system uterus	*No. examined...*	*47*	*46*	*50*
Haemangioma		0	0	1
	Total no. of mice examined...	*48*	*47*	*50*
	No. of tumour-bearing mice...	23	22	16
	No. with multiple tumours...	3	3	3
	No. with malignant tumours...	16	12	5

percentage mortality for the 0-, 75- and 500-ppm exposure levels being 17, 19 and 13, respectively, at wk 52, 40, 32 and 35, respectively, at wk 72.

Histopathological evaluation of the tissues of the female mice revealed a number of age-related changes. A high background incidence of respiratory disease was seen in all groups. This made the interpretation of the findings of the respiratory tract difficult to assess. However, there was no evidence of any treatment-related non-neoplastic effects in these or other tissues examined. The neoplastic lesions were of low incidence in this study. The only tumours seen in the respiratory tract were lung adenomas, which were seen at similar rates of incidence in test and control animals, and one osteosarcoma in the nasal sinus of one animal at the 75-ppm level. The incidence, localization and malignancy of these and the other types of tumours (Table 3) did not indicate any carcinogenic activity of *p*-DCB at levels up to 500 ppm in the female mice.

Effects on reproduction

The long-term inhalation studies on male and female rats and female mice, described above, did not reveal any evidence of pathological changes in the reproductive and accessory tissues (Riley *et al.* 1980a; Riley, Chart, Gaskell & Gore, 1980b). Moreover during a dominant lethal assay on mice, involving vapour exposure levels of 75, 225 and 450 ppm *p*-DCB (6 hr/day for 5 days), there was no treatment-related reduction in the percentage of male mice successfully impregnating females (Anderson & Hodge, 1976).

An embryotoxicity and teratogenicity study on rats using the inhalation exposure route has been performed by Hodge, Palmer, Wilson & Bennett (1977). During that study, groups of at least 20 pregnant SPF rats were exposed to *p*-DCB for 6 hr/day from day 6 to 15 (inclusive) of pregnancy to atmospheric concentrations of 0 (air control), 75, 200 or 500 ppm. The

exposure concentrations were monitored and recorded.

During the experiment, the animals were observed for clinical signs. Body weights were recorded on days 0, 6, 10, 16 and 21 of pregnancy. During caesarean section on day 21 the intact uterus was inspected for numbers of viable foetuses, their sex and weight being recorded. Resorptions and corpora lutea were also observed and noted. The foetuses were examined externally, and then inspection of the skeletal system was made on alternative foetuses after evisceration. The remaining foetuses were subjected to examination by Wilson's technique (Wilson, 1965).

Maternal weight gain during pregnancy was normal and there were no clinical or pathological signs of toxicity. One dam at the 75-ppm level, one at the 200-ppm level and five dams at the 500-ppm level littered 1 day earlier than expected for this strain.

The exposure to *p*-DCB produced no adverse effects on numbers of implantations, resorptions, viable foetuses, runts or corpora lutea, mean foetal weight, litter weight or sex ratios. There was one foetus with gastroschisis and malrotation of the left hindlimb in the 75-ppm group, one with gastroschisis and malrotation of the right hindlimb at 200 ppm, and one with agnathia and cleft palate at 500 ppm, while one foetus among the control animals was found to be anaemic with excessive haemorrhage after severance of the umbilical cord. There was no evidence of retarded ossification or increased incidence of minor skeletal variants at any exposure level. Neither did soft-tissue examination indicate any treatment-related changes.

It was therefore concluded that there was no evidence of embryo- or foetotoxicity, nor any indication of a teratogenic effect at levels up to and including 500 ppm *p*-DCB.

Mutagenicity

In vitro *systems*

The mutagenic potential of *p*-DCB was assayed in the *Salmonella typhimurium* mutagenicity assay (Ames test; Anderson, 1976) using TA1535, TA1538, TA98 and TA100 strains with and without liver post-mitochondrial supernatant (S-9 mix) from rats pretreated with Aroclor. The bacteria were exposed to gaseous concentrations of *p*-DCB of 682, 299 and 94 ppm in air or to *p*-DCB dissolved in dimethylsulphoxide (DMSO) at concentrations of 2500, 500, 100, 20 and 4 μg/plate.

These conditions were used in a series of three experiments, one with *p*-DCB in the gaseous phase and two with *p*-DCB dissolved in DMSO. There were no biologically significant increases in the Salmonella revertant colony above negative control values in any strain at any level of gaseous exposure. In the two experiments using *p*-DCB dissolved in DMSO, one strain only (TA1535) showed a more than twofold increase in colony numbers at a concentration of 500 μg/plate in the presence of S-9 in one of the experiments.

The data indicate that *p*-DCB was not mutagenic under the experimental conditions except in one strain, TA1535, as seen in the second series of experiments. However, this effect was not reproducible in the other experiments of this series.

The lack of effect with S-9 mix indicates that *p*-DCB was not activated to a mutagenic metabolite. The overall lack of any dose-related mutagenic effect was not due to the insensitivity of the test system, since there was a mutagenic response with each of the positive control substances (2-nitrofluorene and 2-(1-chloro-2-isopropylaminoethyl)naphthalene).

In vivo *systems*

Dominant lethal assay. The mutagenic activity of *p*-DCB at three exposure levels has been assessed in fertile male CD-1 mice by the dominant lethal test (Anderson & Hodge, 1976). Groups of 16 male mice were exposed by inhalation to *p*-DCB at 75, 225 or 450 ppm for 6 hr/day for 5 days. A group of 35 control males was exposed to air. After the exposure period, test-mating with untreated females was started. The males were mated with new virgin females each week for 8 wk. Females were killed 13 days after fertilization and the uteri were examined for live implantations and for early and late deaths.

There was no significant increase in post-implantational early foetal deaths, as shown by the mean number of early deaths per pregnant female. There was also no significant increase in early deaths as shown by the number of females with one or more early deaths, except at wk 1 in the group exposed to 225 ppm. Similarly, there was no increase in the incidence of early deaths expressed as a percentage of total implantations per pregnancy, except in wk 6 in the group exposed to 225 ppm. There was no evidence in wk 1–7 of a pre-implantational egg loss, as indicated by the total implantations per pregnant female. In wk 8 there were significant decreases in the groups exposed to 75 and 450 ppm *p*-DCB, but since there was no corresponding increase in the number of early deaths in these weeks, the observed decreases in total implantations were probably due to other than mutagenic factors.

Fertility was measured by the percentage of male mice successfully mating each week or the percentage of female mice that became pregnant. No reduction in fertility was observed except in wk 6 and 7 in the group exposed to 75 ppm. Since these findings were due to the relatively high level of the negative control in those weeks, and as there was no dose-response relationship, these lowered values were not considered biologically important.

Thus, *p*-DCB was not mutagenic in the dominant lethal test at any maturation stage of the 8-wk spermatogenic cycle in mice exposed to levels up to 450 ppm. Positive responses were produced in concurrent positive control groups using males treated with cyclophosphamide (200 mg/kg ip), ethyl methanesulphonate (150 mg/kg orally) or nitrogen mustard (2.5 mg/kg ip).

Cytogenetic assay. Cytogenetic investigations were carried out on the bone-marrow cells of rats (Alderley Park strain) exposed to *p*-DCB by inhalation (Anderson & Richardson, 1976). Four animals served as controls and groups of three rats were treated in one of the following ways:

(i) by a single 2-hr exposure at concentrations of 299 and 682 ppm;

(ii) by multiple exposure for 5 hr/day on five successive days at concentrations of 75 and 500 ppm; (iii) By multiple exposure for 5 hr/day on 5 days/wk for 3 months, also at concentrations of 75 and 500 ppm.

The animals were killed 22 hr after exposure, bone-marrow samples were prepared according to the method of Sugiyama & May (1971) and 50 or 100 cells from each animal were investigated. Abnormalities were assigned to the following four categories: chromatid or chromosome gaps, chromatid breaks, fragments and any other complex abnormality.

No increase in the number of observable chromosome abnormalities in rat bone-marrow cells could be detected at exposure levels as high as 682 ppm *p*-DCB. However, significant differences from the negative control groups were found with the positive control substances, benzene and vinyl chloride.

Metabolism, distribution and elimination

Earlier studies on the metabolism of *p*-DCB have been discussed by Hawkins, Chasseaud, Woodhouse & Cresswell (1980), who themselves studied tissue levels and the kinetics of excretion of *p*-DCB in rats by monitoring ^{14}C activities following administration of labelled *p*-DCB by inhalation (1000 ppm, 3 hr day) or by the oral or subcutaneous routes (250 mg/kg) for up to 10 days. Absorption of *p*-DCB was high by the oral and inhalation routes and prolonged after subcutaneous injection. Highest radioactivities were detected in fat, kidneys, liver and lungs. A similar distribution pattern was observed in these tissues, regardless of the mode of exposure. Most of the ^{14}C activity was eliminated in the urine (91–97%) within 5 days, while small amounts were found in the faeces or in expired air. However, 50–60% was excreted in the bile during 2 days. Much of this material must have been reabsorbed (enterohepatic circulation) and ultimately excreted in the urine after polar metabolites of higher molecular weight had been formed. There were only quantitative differences in the components eliminated via urine and bile. The major components were a sulphate of 2,5-dichlorophenol and to a lesser extent a glucuronide conjugate of the same phenol. Minor components were identified as a dichloroquinol and a mercapturic acid of *p*-DCB. Oxidation was postulated to be involved in the first step of the degradative pathway (see also Reid & Krishna, 1973).

Excretion after exposure to *p*-DCB by either route was more rapid after repeated dosage, an observation in line with the finding of several workers that *p*-DCB has an inducing effect on various metabolizing enzymes (Carlson & Tardiff, 1976; Reid & Krishna, 1973; Townsend & Carlson, 1981). Hawkins *et al.* (1980) found that 5 days after cessation of exposure, ^{14}C activities declined to near or below the limit of detection (<0.2 ppm).

In man *p*-DCB has been found to be metabolized to phenolic compounds which are eliminated as glucuronic or sulphuric acid conjugates (Hallowell, 1959; Pagnotto & Walkley, 1965). Additional sulphur-containing metabolites of *p*-DCB were found in a single-dose study in which 200 or 800 mg/kg was administered orally to rats (Kimura, Hayashi, Sato *et al.* 1979). Besides the major component 2,5-dichlorophenol, minimal amounts of 2,5-dichlorophenylmethylsulphoxide and -sulphone were identified in tissue and urine samples. The mechanism for the formation of these compounds needs further investigation.

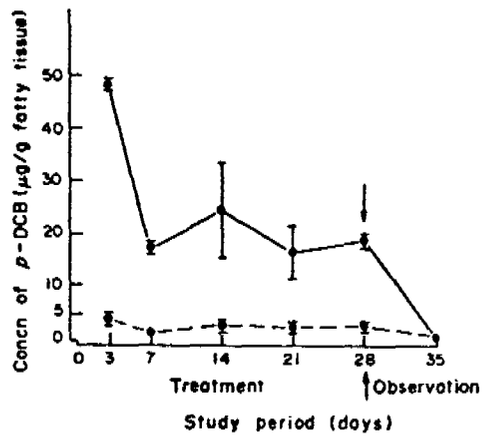

Fig. 1. Concentration of *p*-dichlorobenzene (*p*-DCB) in the fatty tissue of rats during and for 1 wk after the feeding of diet containing 100 (---) or 1000 (——) ppm *p*-DCB for 28 days. Points and bars indicate means ± SEM for groups of three rats. Arrows mark the transition from treatment to the observation period.

Similar evidence for rapid elimination and lack of accumulation was found by Schmidt & Loeser (1977), who administered *p*-DCB to rats (i) orally as a single dose of 100 or 1000 mg/kg, (ii) at a level of 100 or 1000 ppm in diet fed for a period of 28 days or (iii) by inhalation over 76 wk at concentrations of 75 or 500 ppm followed by a 36-wk observation period (long-term inhalation study described above). In all three studies, *p*-DCB and the primary metabolite 2,5-dichlorophenol were determined by GC-ECD after enzymatic splitting of conjugates in plasma, urine, fatty tissue, liver and kidneys. In the acute study, all concentration curves showed rapid (2–4 days) decline in all tissues and plasma and 40–60% of the dose was eliminated as 2,5-dichlorophenol conjugates in the urine. In the feeding study concentrations in all tissues declined from day 3 to day 7, after which *p*-DCB levels maintained a steady state during the rest of the feeding period (Fig. 1). One week after cessation of *p*-DCB intake neither *p*-DCB nor 2,5-dichlorophenol components were detectable in any tissue. During the inhalation exposure, tissue levels were found to be lower after 76 wk than after 26 wk demonstrating a decline to the end of the exposure. Neither *p*-DCB nor its major metabolite could be detected in fatty tissue 36 wk after cessation of inhalation.

Conclusions

The chronic toxicity data of Hollingsworth *et al.* (1956) demonstrate that exposure of several species to high concentrations (approximately 800 ppm) of *p*-DCB induced higher liver weights as well as some minor histological changes. A level of about 100 ppm, however, did not have any influence on the liver. The liver as a target organ after high oral or vapour

exposure seems to have been established also by other studies involving either acute or short-term treatment (Frada & Cali, 1959; Totaro, 1961; Zupko & Edwards, 1949). The effects, however, seem to be mild and are in contrast to the severe effects seen after *o*-dichlorobenzene treatment (Koch-Weser, de la Huerga, Yesinick & Popper, 1953; Reid, 1973; Reid & Krishna, 1973).

Differences in covalent binding to macromolecules and dependence on glutathione have been discussed as reasons for the differences in hepatotoxicity between the two isomers. The long-term studies in rats and mice detailed in this report failed to demonstrate hepatotoxicity even at high-level (500-ppm) exposure to *p*-DCB. Higher liver weights in the 500-ppm group accompanied by a slightly elevated coproporphyrin output suggest some minor effects on liver function. No histological changes or increase in hepatic aminopyrine demethylase were detected however. Higher kidney weights at this level are only suggestive of a minor influence. Earlier acute-exposure studies in several animal species demonstrated more severe effects on the kidney than on the liver (Zupko & Edwards, 1949). However, this has not been substantiated by several other short- and long-term studies, including those presented in this paper.

There is no evidence from the long-term inhalation studies in rats that *p*-DCB induces haematological changes. Short-term repeated inhalation studies by Zupko & Edwards (1949) produced evidence of granulocytopenia, which disappeared during post-treatment periods. The long-term exposure of rats to levels up to 500 ppm did not result in changes in the peripheral blood or the bone marrow. The results by Zupko & Edwards (1949) were not confirmed, therefore, even after rather long exposure periods.

With respect to carcinogenicity, the long-term rat and female mouse studies did not demonstrate an excess of tumours, which would be indicative of a carcinogenic response, after inhalation exposure to levels up to 500 ppm ($=3000$ mg/m^3 air). A suggested association between leukaemias in workers and exposure to chlorobenzenes including *p*-DCB (Girard, Tolot, Martin & Bourret, 1969) was considered by an IARC Working Group (1974) to be insufficient evidence on which to assess a carcinogenic risk. The results of the long-term rodent studies reviewed in this paper support the view that *p*-DCB has no carcinogenic potential.

The data have not demonstrated any mutagenic activity in a number of *S. typhimurium* tester strains in non-activated and metabolically activated systems. The results were negative after vapour exposure and after direct addition of *p*-DCB in solution to the plates. Lawlor, Haworth & Voytek (1979) similarly failed to demonstrate higher incidences of back mutations in *S. typhimurium* using the liquid suspension technique. They reported positive initial results on some DNA repair-deficient tester bacteria with some chlorinated benzenes. However, the compounds indicating positive responses were not specified.

Several other *in vitro* systems using *Aspergillus nidulans* (Prasad, 1970), HeLa cells (Myhr, 1973) and root tips of different plants (Sarbhoy, 1980; Sharma & Agarwal, 1980; Srivastava, 1966) were used to evaluate the possible mutagenic potential of *p*-DCB. These systems did indicate mutagenic activity. The interpretation of the results reported is difficult, however, since no confirmatory results are available on these test systems. Also no correlation of the results with carcinogenic effects has been established, nor has the use of these systems as an index for mutagenic potential in higher organisms been confirmed. A series of *in vivo* mutagenicity tests using the dominant lethal assay in mice and a cytogenetic assay on the bone marrow of rats demonstrated no effect suggestive of *p*-DCB mutagenicity. Therefore, it is concluded that there is no evidence at present that would indicate that *p*-DCB is a potential mutagen to animals and man.

Neither the dominant lethal nor the embryotoxicity studies using concentrations as high as 500 ppm *p*-DCB in air showed any evidence for an effect on reproductive functions. The slightly earlier littering of a few dams in the treated groups of the teratology study was considered to be incidental and unrelated to *p*-DCB exposure. The absence of any higher incidence of minor or major changes in the foetuses indicated a lack of teratogenic potency of *p*-DCB in the test system used.

As demonstrated in metabolic studies, *p*-DCB is rapidly absorbed and distributed to various tissue components. *p*-DCB and its major metabolite, 2,5-dichlorophenol, can be detected in fatty tissues, liver, kidney and blood after exposure by the subcutaneous, oral or inhalation route (Hawkins *et al.* 1980; Kimura *et al.* 1979; Schmidt & Loeser, 1977). No accumulation has been observed after subacute or long-term exposure. The tissue levels even decline during continuous exposure periods (Hawkins *et al.* 1980; Schmidt & Loeser, 1977). Rapid elimination has been demonstrated after cessation of exposure.

The low order of toxicity of *p*-DCB may be due to its oxidation to phenolic products and then the rapid formation of mainly sulphuric acid and glucuronic acid conjugates (Hawkins *et al.* 1980).

In conclusion, the overall evaluation of the data from recent studies on *p*-DCB shows that it is neither teratogenic nor mutagenic in animals. Long-term studies have shown evidence for only a mild effect on liver function at 500 ppm *p*-DCB in the rat and no toxicological effect at 75 ppm. There was no evidence of carcinogenic potential in the rodent in these studies. Therefore, the experimental evidence justifies the maintenance of the current permissible exposure level.

Acknowledgement—The studies presented here were initiated and sponsored by an industry group on *p*-DCB (Bayer AG, ICI, Rhone Poulenc and Ugine Kuhlmann).

REFERENCES*

American Conference of Governmental Industrial Hygienists (1982). TLVs, Threshold Limit Values for Chemical Substances in Work Air Adopted by ACGIH for 1982. ACGIH, Cincinnati, OH.

Anderson D. (1976). Paradichlorobenzene: Estimation of its Mutagenic Potential in the *Salmonella typhimurium* Plate Incorporation Mutagenicity Assay. Unpublished report:

*The unpublished company reports cited in this list can be made available upon request to the company concerned.

Imperial Chemical Industries Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.

Anderson D. & Hodge M. C. E. (1976). Paradichlorobenzene: Dominant Lethal Study in the Mouse. Unpublished report: Imperial Chemical Industries Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.

Anderson D. & Richardson C. R. (1976). Paradichlorobenzene: Cytogenetic Study in the Rat. Unpublished report: Imperial Chemical Industries Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.

Carlson G. P. & Tardiff R. G. (1976). Effect of chlorinated benzenes on the metabolism of foreign organic compounds. *Toxic. appl. Pharmac.* **36**, 383.

Frada G. & Cali V. (1959). Azione tossica del paradiclorobenzene. *Folia med., Napoli* **41**, 349.

Girard R., Tolot F., Martin P. & Bourret J. (1969). Hémopathies graves et exposition à des dérivés chlorés du benzène (à propos de 7 cas). *J. Méd. Lyon* **50**, 771.

Hallowell M. (1959). Acute haemolytic anaemia following the ingestion of para-dichlorobenzene. *Archs Dis. Childh.* **34**, 74.

Hawkins D. R., Chasseaud L. F., Woodhouse R. N. & Cresswell D. G. (1980). The distribution, excretion and biotransformation of *p*-dichloro[^{14}C]benzene in rats after repeated inhalation, oral and subcutaneous doses. *Xenobiotica* **10**, 81.

Hodge M. C. E., Palmer S., Wilson J. & Bennett I. P. (1977). Para-dichlorobenzene: Teratogenicity Study in Rats. Unpublished report: Imperial Chemical Industries Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.

Hollingsworth R. L., Rowe V. K., Oyen F., Hoyle H. R. & Spencer H. C. (1956). Toxicity of paradichlorobenzene. *Archs ind. Hlth* **14**, 138

IARC Working Group (1974). *Ortho-* and *para-*Dichlorobenzene. In *Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Vol. 7. Some Anti-thyroid and Related Substances, Nitrofurans and Industrial Chemicals.* p. 231. International Agency for Research on Cancer, Lyon.

Kimura R., Hayashi T., Sato M., Aimoto T. & Murata T. (1979). Identification of sulfur-containing metabolites of p-dichlorobenzene and their disposition in rats. *J. Pharmacobio-dyn.* **2**, 237.

Koch-Weser D., de la Huerga J., Yesinick C. & Popper H. (1953). Hepatic necrosis due to bromobenzene as an example of conditional amino acid deficiency. *Metabolism* **2**, 248.

Langhorst M. L. & Nestrick T. J. (1979). Determination of chlorobenzenes in air and biological samples by gas chromatography with photoionization detection. *Analyt. Chem.* **51**, 2018.

Lawlor T., Haworth S. R. & Voytek P. (1979). Evaluation of the genetic activity of nine chlorinated phenols, seven chlorinated benzenes and three chlorinated hexanes. *Envir. Mutagen.* **1**, 143.

McKinney J. D., Fishbein L., Fletcher C. E. & Barthel W. F. (1970). The electron-capture gas chromatography of paradichlorobenzene metabolites as a measure of exposure. *Bull. envir. Contam. Toxicol.* **5**, 354.

Myhr B. C. (1973). A screen for pesticide toxicity to protein and RNA synthesis in HeLa cells. *J. agric. Fd Chem.* **21**, 362.

Pagnotto L. D. & Walkley J. E. (1965). Urinary dichlorophenol as an index of para-dichlorobenzene exposure. *Am. ind. Hyg. Ass. J.* **26**, 137.

Prasad I. (1970). Mutagenic effects of the herbicide 3',4'-dichloropropionanilide and its degradation products. *Can. J. Microbiol.* **16**, 369.

Reid W. (1973). Relationship between tissue necrosis and covalent binding of toxic metabolites of halogenated aromatic hydrocarbons. In *Pharmacology and the Future of Man.* Proceedings of the 5th International Congress of Pharmacology, San Francisco, 1972. Vol. 2, p. 62. S. Karger, Basel.

Reid W. D. & Krishna G. (1973). Centrolobular hepatic necrosis related to covalent binding of metabolites of halogenated aromatic hydrocarbons. *Expl molec. Path.* **18**, 80.

Riley R. A., Chart I. S., Doss A., Gore C. W., Patton D. & Weight T. M. (1980a). Para-dichlorobenzene: Long Term Inhalation Study in the Rat. Unpublished report: Imperial Chemical Industries Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.

Riley R. A., Chart I. S., Gaskell B. & Gore C. W. (1980b). Para-dichlorobenzene: Long Term Inhalation Study in the Mouse. Unpublished report: Imperial Chemical Industries Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.

Sarbhoy R. K. (1970). Effect of paradichlorobenzene on the somatic chromosomes and mitosis of Lens esculenta (L.) moench. *Cytologia* **45**, 381.

Schmidt U. (1977). Method of Determining 1,4 Dichlorobenzene and 2,5 Dichlorophenol in Biological Material by Means of Gas Chromatography. Unpublished report: Bayer AG, Toxicology Institute, 5600 Wuppertal 1, Federal Republic of Germany.

Schmidt U. & Loeser E. (1977). Studies of the Absorption, Distribution and Elimination of 1,4 Dichlorobenzene After Once-only Administration and in the Feeding Test with Rats. Unpublished report: Bayer AG, Toxicology Institute, 5600 Wuppertal 1, Federal Republic of Germany.

Sharma Y. P. & Agarwal K. (1980). Cytomorphological studies on *p*-dichlorobenzene treated maize. *Sci. Cult., Calcutta* **46**, 153.

Srivastava L. M. (1966). Induction of mitotic abnormalities in certain genera of tribe Vicieae by paradichlorobenzene. *Cytologia* **31**, 166.

Sugiyama T. & May B. (1971). Specific vulnerability of the largest telocentric chromosome of rat bone marrow cells to 7,12-dimethylbenz[*a*]anthracene. *J. natn. Cancer Inst.* **47**, 1267.

Totaro S. (1961). Le transaminasi e l'aldolasi sieriche nella intossicazione spermientale subacuta da paradiclorobenzene. *Folia med., Napoli* **44**, 586.

Townsend B. A. & Carlson G. P. (1981). Effect of halogenated benzenes on the toxicity and metabolism of malathion, malaoxon, parathion, and paraoxon in mice. *Toxic. appl. Pharmac.* **60**, 52.

Wilson J. G. (1965). *Teratology: Principles and Techniques* Edited by J. G. Wilson & J. Warkany. University of Chicago Press, Chicago.

Zupko A. G. & Edwards L. D. (1949). A toxicological study of p-dichlorobenzene. *J. Am. pharm. Ass.* **38**, 124.