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A review of recent literature on the Toxicology of Benzene

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A REVIEW OF RECENT LITERATURE ON

THE TOXICOLOGY OF BENZENE

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A. SUMMARY

The Health and Safety Executive (1982) in the UK and the Conseil Européen des Fédérations de l'Industrie Chimique (CEFIC, 1983) have recently reviewed the toxicology of benzene. Since these reports were issued a number of publications have appeared which need to be assessed. Such an assessment is made by ECETOC in this Technical Report.

Papers on the short- and long-term toxicity of benzene, comprising studies of the exposure of rats and mice by gavage and inhalation, are reviewed. It is concluded that benzene is a carcinogen to rats and mice under the conditions of the experiments.

Numerous recent publications on the induction of chromosomal damage by benzene in tissues from rats and mice (in vivo), and chinese hamsters and human lymphocytes (in vitro), confirm that benzene is clastogenic and suggest that at least part of its activity may be due to its metabolites. Recent cytogenetic studies of lymphocytes from workers occupationally exposed to benzene showed no consistently significant elevation in chromosomal aberrations which can be related directly to such exposure. No relationship between the types of chromosome damage observed and effects of exposure to benzene on human health can be established at present.

Several epidemiological studies have appeared recently. The Task Force notes that while there is adequate evidence that exposure to high concentrations of benzene is associated with haematotoxicity and acute myelocytic leukaemia in humans, the question of whether benzene can cause an increased incidence of other forms of lymphopoietic or haematopoietic cancers remains unresolved. It is likely to remain unresolved under the conditions of modern benzene-using operations where the numbers exposed, and the degree of exposure, are low.

Such reports as have been published on the effects of exposure to benzene on human reproduction are contradictory and inconclusive. There is no reliable evidence to suggest any association between exposure to benzene and adverse effects on human reproduction.

The papers published so far on the consequences for human health of exposure to benzene are, without exception, deficient in quantitative exposure data.

Without such data it is impossible to describe the shape of the dose-response curve relating exposure with any of the recorded toxic effects. If future epidemiological studies are to increase our understanding of the adverse effects of benzene on human health they must meet the requirements specified in this report. Little is to be gained in continuing to report the mortality or morbidity of populations exposed to benzene under inadequately-defined conditions.

The recent studies reviewed in this report contribute no new medical or toxicological evidence which justifies amending current benzene exposure standards.

B. INTRODUCTION

Benzene is an important, large-tonnage industrial chemical to which a substantial number of people are exposed. The toxicology of benzene has been reviewed recently by the UK Health and Safety Executive (1982) and CEFIC (1983) in documents which cover the literature up to about 1980 or 1981. Since these reviews were published a number of papers on the animal toxicology of benzene and its effects on human health have appeared, and they are assessed in this report.

In general, this review is restricted to publications which are not dealt with in the above two documents, but some reference back to earlier papers was occasionally necessary.

C. SHORT AND LONG TERM ANIMAL TOXICOLOGY

(The nomenclature for the variety of blood disorders, including various forms of leukaemia, mentioned in this report is somewhat complex, and synonyms are sometimes used for the same disease. Therefore, the terminology of the cited authors is used in the text and a Glossary of Terms has been included in Appendix 2).

This section deals mainly with papers which are most relevant to human exposure and current exposure standards, i.e. to studies on the effects of benzene on experimental animals exposed by inhalation. Some studies on oral exposure are, however, also discussed.

1. Short Term Toxicology

In inhalation studies on immunofunction and haematotoxicity, Baarson et al. (1984) exposed male C57B1 mice to 10 ppm of benzene, 6h/d, 5d/w, for up to 178 days, and Rozen et al. (1984) exposed mice of the same strain and sex to 10, 30, 100 and 300 ppm for 6h/d, for 6 days.The authors concluded that benzene in concentrations around the current occupational exposure limit (10 ppm) may have an influence on immunofunction and may be haematotoxic. This conclusion is based on i) the observed depression in the number of splenic nucleated erythrocytes, and of circulating erythrocytes and lymphocytes, and ii) the depression of the in vitro colony-forming ability of erythroid progenitor cells and depression of in vitro colony mitogen-induced blastogenesis of femoral B- and splenic T-lymphocytes.

Detailed data, eg. numbers of femoral erythroid cells, colony-forming ability of femoral T-lymphocytes and splenic B-lymphocytes, and the differential count of the lymphocyte population, are not given in the papers. There is no critical analysis of such important factors as dose-reponse relationships, trends with time, statistical significance or any limitations of the techniques used. This makes the evaluation of the results difficult. Some of the numerical data vary over a wide range, and it seems questionable whether these results could be reproduced. There is little experience of the biological relevance of certain of the changes in immune cell functions as detected by the techniques used, and these techniques need to be validated by studying a range of chemicals.

In a study for the American Petroleum Institute (Hazelton Labs., 1983), male and female CD-1 mice and SD-CD rats were exposed, by inhalation, to 0, 1, 10, 30 and 300 ppm of benzene, 6 h/d, 5 d/w for 90 days. At 300 ppm, testicular, ovarian and bone marrow damage were found in mice. Gonadal damage included testicular atrophy and degeneration, with a decrease in the number of spermatozoa in the epididymal ducts and an increase in the incidence of abnormal sperm forms. Four out of forty females had bilateral ovarian cysts. At 1, 10 and 30 ppm, only minimal testicular and ovarian effects of doubtful biological significance were observed, in the mice only.

Exposure of rats to 300 ppm resulted in lower body weights, decreased total leukocyte and lymphocyte counts, and increased neutrophil counts. A tendency

towards decreased cellularity in the femoral bone marrow was seen in the same group. At lower doses no treatment-related effects were observed in rats.

Toft et al.(1982) found that when NMRI mice were exposed either continuously during 4-10 days, or intermittently (8 h/d, 5 d/w for 2 weeks), to concentrations of benzene in air ranging from 1 to 200 ppm, haematotoxic effects occurred at 20 ppm. The effects were on nucleated red cells, and the colony-forming granulopoietic stem cells, of the tibia.

2. Long Term Toxicology

2.1. Oral exposure

2.1.1. Maltoni et al.(1982a, 1982d, 1983). These reports relate to a single study in which a dose of 500 mg/kgbw of benzene dissolved in olive oil was administered to 40 male and 40 female Sprague-Dawley rats, by stomach tube, once per day on 4 or 5 d/w. The study was intended to run for 104 weeks. Control groups of 50 males and 50 females received olive oil only. The rats were 7 weeks old at the start of the study and were observed until they died spontaneously or had to be sacrificed in a moribund state. Gross observations and body weights were recorded, and some haematological examinations on circulating blood were performed on a limited number of rats at week 84. Standard autopsies and histopathology were carried out on sacrificed rats or on those found dead.

In the 1983 paper, results are reported at 92 weeks. Exposure to benzene correlated with slightly lowered body weights in both sexes, more marked in females than in males, and with reduced red and white blood cell counts which were mostly due to lymphocytopenia. Survival at week 92 was higher in the exposed males (72.5%) than in the controls (58%), but slightly lower for the females (60, against 66% in the controls). The incidence of Zymbal gland carcinomas was 15% in both exposed males and females, and one occurred in a male of the control group. This tumour has been reported to occur at a frequency of 0.9% in the strain of rat used (Maltoni and Scarnato, 1983).

Carcinomas of the oral cavity orginating mainly at the lips were observed with incidences of 17.5 and 10% in males and females, respectively, and were zero in the controls and historical controls. One

carcinoma in the nasal cavity of a treated male was also reported. One unspecified type of leukaemia was observed in a female rat of the control group and in one of the dosed animals, but neither the bone marrow nor lymphnodes was examined. One angiosarcoma of the liver was observed in a female of the exposed group.

2.1.2. Maltoni and Scarnato (1979, 1983) reported a similar study on groups of 30 male and 30 female Sprague-Dawley rats (dose : 50 mg/kgbw) and 35 of each sex (dose : 250 mg/kgbw), with 30 animals of each sex in control groups receiving olive oil only. Treatment up to week 52 was followed by an observation period until the animals died. In this study no haematology, or histopathology of the lymphnodes or bone marrow, was reported. The tumour incidences at week 144 (termination of the study) are shown in Table 1.

TABLE 1

Incidence of Tumours in Sprague-Dawley Rats in Study by Maltoni and Scarnato (1979, 1983)

	Control		50 mg/kgbw		250 mg/kgbw	
	M	F	M	F	M	F
Zymbal Gland Carcinoma	0/28	0/30	0/28	2/30	0/33	8/32
Leukaemia	0/28	1/30(a)	0/28	2/30(b)	4/33(c)	1/32(d)
Carcinoma of oral cavity	0/28	0/30	0/28	0/30	0/33	2/32
Mammary Carcinoma	0/28	3/30	0/28	4/30	0/33	7/32
Angiosarcoma	0/28	0/30	0/28	0/30	1/33(e)	0/32
Hepatoma	0/28	0/30	0/28	0/30	1/33	0/32

(a) haematosarcoma
(b) 1 myelogenous leukaemia, 1 haematosarcoma
(c) 1 lymphoblastic leukaemia, 3 haematosarcomas
(d) 1 lymphocytic leukaemia
(e) subcutaneous

Apart from the results reported above, the authors stated that benzene "did not show acute or sub-acute toxic effects". No data were provided on time-related mortality or time-of-occurrence of the tumours.

2.1.3. NTP study. Two-year toxicology and carcinogenicity studies with oral dosing have been performed on F344/N rats and B6C3F$_1$ mice under the US National Toxicology Programme (NTP, 1984).

a) Rat study. Doses of 0, 50, 100 and 200 mg/kgbw of benzene in corn oil (5 ml/kg) were administered by gavage to groups of 50 male rats for 5 d/w over 103 weeks. Groups of 50 female rats were similarly administered 0, 25, 50 and 100 mg/kgbw. Ten additional animals were placed in each group for interim sacrifice at 12 months.

Daily observations on the health status of the animals were made. Haematological analyses (HCT, RBC, WBC, differential WBC, reticulocyte count, prothrombin time, haemaglobin and MCV) were performed on 10 animals from each sex and group at 12, 15, 18 and 21 months from the start of the study. These analyses were also performed on the additional animals selected for interim sacrifice after 0, 3, 6, 9 and 12 months exposure. Rats killed in extremis, found dead, or sacrificed at the end of the study were autopsied and detailed histopathological investigations were carried out.

The mean body weight of the male rats in the 200 mg/kgbw group at week 103 was 23% lower than that of the controls administered corn oil only. The survival in all exposed groups was lower than that of the controls, and was dose-dependent :

	Controls	25	50	100	200 mg/kgbw
males	32/50	-	29/50	25/50	16/50
females	46/50	38/50	34/50	25/50	-

Effects on the haematopoietic system, Zymbal gland, stomach, adrenals, skin and oral cavity were found. An increased incidence of lymphoid depletion of the splenic follicles was observed in males and females. Dose-related leukopenia occurred in both sexes during the first 18 months of the study. Zymbal gland carcinomas were found in the exposed groups of both sexes at a higher incidence than in the controls :

	Controls	25	50	100	200 mg/kgbw
males	2/50	-	6/50	10/50*	17/50*
females	0/50	5/50*	5/50*	14/50*	-

(Detailed statistical analyses by various methods are given in the NTP paper, and throughout this section * indicates a statistically-significant difference between the exposed and control animals as determined by the Fisher test, $p<0.05$).

The incidence of epithelial hyperplasia of the Zymbal gland was also higher than that of the controls for the mid- and high-dose males, and high-dose females.

	Controls	25	50	100	200 mg/kgbw
males	0/49	-	3/48	12/50*	10/49*
females	1/49	1/45	2/50	6/49*	-

Exposure to benzene was associated with increased incidences of neoplasms of the skin (males only) and oral cavity. The incidences of squamous cell papillomas and squamous cell carcinomas of the skin were :

	Controls	50	100	200	mg/kg/bw
males	0/50	2/50	1/50	5/50	(papillomas
males	0/50	5/50	3/50	8/50*	carcinomas)

Squamous cell papillomas and squamous cell carcinomas of the oral cavity were observed in exposed rats at incidences which were statistically significantly higher than in the controls for both types, separately and combined. The incidences for both types combined were :

	Controls	25	50	100	200 mg/kgbw
males	1/50	-	9/50*	16/50*	19/50*
females	1/50	5/50*	12/50*	9/50*	-

b) Mouse study. Doses of 0, 25, 50 and 100 mg/kgbw of benzene were administered to male and female mice by gavage, for 5d/w over 103 weeks, in a study conducted with a protocol similar to that used for rats. Mean body weights of the mice in the 100 mg/kgbw group were 14

and 17% less than those of the controls, for males and females respectively. There was no effect on body weight at lower levels. The survival of dosed animals was lower than that of the controls, and was dose-dependent, as shown by the mortality figures:

	Controls	25	50	100	mg/kgbw
males	7/50	18/50	23/50	28/50	
females	18/50	24/50	26/50	30/50	

Effects on the haematopoietic system, Zymbal gland, stomach and adrenals were found (as in rats), but there were no lesions of the oral cavity in the mice. The incidence of malignant lymphomas was higher than that in the controls for all treated groups :

	Controls	25	50	100	mg/kgbw
males	4/49	9/48*	9/50*	15/49*	
females	15/49	24/45*	24/50*	19/49*	

Bone marrow haematopoietic hyperplasia was observed at increased incidences in both sexes :

	Controls	25	50	100	mg/kgbw
males	0/49	11/48*	10/50*	25/49*	
females	3/49	14/45*	8/50*	13/49*	

Dose-related leukopenia was observed in both sexes in the first 18 months only. Zymbal gland carcinomas were of higher incidence than in the controls in the case of the mid- and high-dose male mice and the high-dose female mice :

	Controls	25	50	100	mg/kgbw
males	0/49	1/48	4/50*	21/49*	
females	0/49	0/45	1/50	3/49*	

In mid- and high-dose males, and high-dose females, the incidence of epithelial hyperplasia of the Zymbal gland was also increased :

	Controls	25	50	100	mg/kgbw
males	0/49	3/48	12/50*	10/49*	
females	1/49	1/45	2/50	6/49*	

In mice of both sexes there was a higher incidence of alveolar epithelial hyperplasia at 50 and 100 mg/kgbw, and alveolar/bronchiolar adenomas and carcinomas were observed at incidences higher than in the controls in the mid- and/or high-dose groups. Other lesions such as focal hyperplasia and adenomas of the Harderian gland, and hyperplasia and squamous cell carcinomas of the preputial gland, were also of higher incidence in the dosed mice. Mammary carcinomas and carcinosarcomas occured at higher incidences in the mid- and/or high-dose groups. Hepatocellular adenomas and carcinomas were also of higher incidence in treated females than in the controls, and were dose-related :

	Controls	25	50	100	mg/kgbw
females	0/49	2/45	5/50*	10/49*	(adenomas)
females	0/49	0/45	1/50	4/49*	(carcinomas)

The authors concluded that there was clear evidence that benzene was carcinogenic to rats and mice.

2.2. Exposure by inhalation

2.2.1. Maltoni et al.(1982b, 1982c and 1983). In these three publications a single study is reported in which 12-day pregnant Sprague-Dawley rats were exposed to benzene at 200 ppm, 4 h/d, for 5 d/w until parturition. The offspring were then exposed to 200 or 300 ppm of benzene as follows:

- a sub-group i) of 70 male and 59 female offspring was exposed to 200 ppm, 4 h/d, 5 d/w for 7 weeks, and then 7 h/d, 5 d/w for 8 weeks;
- a sub-group ii) of 75 male and 65 female offspring was exposed to 200 ppm, 4 h/d, 5 d/w for 7 weeks and then 7 h/d, 5 d/w for 12 weeks followed by an exposure to 300 ppm 7 h/d, 5 d/w for 85 weeks. The total exposure was for 104 weeks.

Groups of 158 males and 149 females were used as controls. After cessation of exposure, the animals were observed for the remainder of

their life. The latest publication (Maltoni et al., 1983) gives the results at week 118, at which time the study was still continuing.

The body weight of the exposed males was slightly affected. Mortality was higher than in the controls, but may be significant only in sub-group (ii). The WBCs tended to be lower in sub-group (ii) after 98 weeks of exposure. There were no exposure-related effects on WBCs or RBCs. The incidence of hepatic pre- and neo-plastic lesions were :

- controls ; no hepatomas, or nodular hyperplasia or dysplasia.
- sub-group (i) ; 3 hepatomas in females only, nodular hyperplasia in 2 males and 7 females.
- sub-group (ii) ; hepatomas in 2 males and 1 female, nodular hyperplasia in 1 male and 5 females, 2 nodular dysplasias in females only.

The incidence of leukaemias was :

- controls ; 1 in a male.
- sub-group (ii); 2 in males.

No Zymbal gland tumours were observed. The exposed parent females developed 1 hepatoma and 5 nodular hyperplasias, whereas none was observed in the control group.

2.2.2. Snyder et al.(1980) exposed groups of 50 AKR/J and 40 C57BL/6J male mice to benzene, by inhalation, at levels of 100 and 300 ppm respectively, for 6h/d, 5d/w for life. The same numbers of animals were used as controls. Clinical observations and body weight were regularly recorded, and haematological studies (RBC, and total and differential WBCs) were performed every second week on 10 exposed and 10 control mice. Gross pathology was carried out on all animals, and routine histopathology on the lung, spleen, liver, kidneys and bone marrow only.

Exposure to 100 ppm had no influence on the survival or body weight gain of the AKR/J strain, but a significant incidence of lymphocytopenia occured at an early stage in the study. RBCs were also depressed, and bone marrow hyperplasia was of significantly higher incidence in the 100 ppm group (10, as against 1 in the controls). No other significant effect was reported for the AKR/J strain.

The body weight gain and survival of C57BL/6J mice exposed to 300 ppm of benzene were lower than in the controls. Lymphocytopenia, RBC depletion, and other haematological changes such as anisocytosis, poikilocytosis, and left shift in differential WBC also occured in the exposed mice. Histological examination showed a higher incidence of haematopoietic neoplasms (controls 2/40; exposed animals 8/40) comprising 2 lymphomas in the controls and 6 in exposed animals, plus 1 myeloma and one leukaemia in the exposed. Bone marrow and spleen hyperplasia (non-neoplastic lesions) were of higher incidence in the exposed than in the control group, the spleen hyperplasia being due mainly to ectopic haematopoiesis.

2.2.3. Goldstein et al. (1982) exposed groups of 40 CD1 mice to 0 and 300 ppm of benzene, 6h/d, 5d/w for 31 weeks (see below), followed by observation for life. One of the mice exposed to 300 ppm developed a chronic myelogenous leukaemia, another an acute myeloblastic leukaemia, and a third had a granulocytic hyperplasia. Two other unspecified tumours were reported. Further details of the same study are given by Snyder et al. (1982) who found that life was shortened at 300 ppm, the exposure therefore being discontinued after 31 weeks. Five neoplasms (2 malignant lymphomas and 2 myeloid leukaemias already reported by Goldstein, plus a benign adenoma) were found in the 300 ppm group, and two neoplasms (malignant lymphomas with no thymic involvement) were found in the controls. Bone marrow hyper- and hypo-plasia, and an increase in splenic haemosiderin pigments, were observed in the 300 ppm group at a statistically-significant higher incidence.

Because the number of surviving animals was low, as was the number of neoplasms (there was no statistically-significant difference between the exposed and control groups) it is difficult to draw conclusions from this study, except that there were clear effects on the haematopoetic system at 300 ppm.

2.2.4. Snyder et al.(1984). Haematological investigations (RBC, and total and WBCs) were made several times within the first 1.5 year of the exposure of male Sprague-Dawley rats (40 controls and 40 exposed) to 100 ppm of benzene by inhalation. Animals found dead, or sacrificed in a moribund condition, were autopsied. Samples from lung, liver, spleen, bone

marrow, and kidney were investigated histologically, as were any abnormalities.

During the study, an epidemic respiratory infection occurred between days 448 and 504, with resulting high mortality (20 controls and 13 exposed rats died). No differences in weight gain between the groups were observed. Erythrocyte and lymphocyte counts of exposed rats were lower than those of the controls but were rarely significant. Eleven exposed rats developed neoplasms, including 1 chronic myelogenous leukaemia, 2 Zymbal gland carcinomas, 1 hepatoma, 1 cholangioma, 1 liver haemangioma, 1 liver haemangio-endothelioma, and 1 fibrosarcoma and squamous cell carcinoma in the facial area. Only 3 tumours (1 fibrosarcoma and one fibroma of the trunk, and 1 osteogenic sarcoma of the mandible) occured in the control animals. The mortality-corrected incidences of both total and malignant tumours in the treated rats were not significantly greater than those in the controls.

The authors concluded from the limited data available, that 100 ppm of benzene was haematotoxic and carcinogenic to rats.

2.3. Discussion and conclusions

The above long-term studies on rats by Maltoni et al., most of which had not been completed at the time of writing, are lacking in a number of requirements which would permit a full evaluation of the results (important details are not given, and the results are not evaluated statistically). The unusual exposure regime in the Maltoni inhalation study also makes interpretation difficult. Although the haematopoietic system is involved in certain of the effects, no historical data are given. Only limited haematological data are reported but there is no detailed description of the methods by which they were determined. Data on bone marrow and lymphnodes are also lacking. The background incidence of tumours is considered in a few cases, but fluctuations in this incidence rather than a single percentage value are important for assessing the results of a specific study.

Regarding Zymbal gland tumours, although the data of Maltoni et al. may reflect only normal fluctuations rather than benzene-related tumours, it is significant that other investigators also reported a higher incidence