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COMMISSION OF THE EUROPEAN COMMUNITIES

THE ACUTE HEPATIC TOXICITY OF ORGANIC REACTOR COOLANTS

by

H. OTT and D. PIRRWITZ

1970

Joint Nuclear Research Center
Ispra Establishment — Italy

Biology Division

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SUMMARY

Liver function in rat after ingestion of single doses of the organic reactor coolants OM–2, DOM, THERMIP and HB–40 and their main components *o*-terphenyl, *m*-terphenyl, diphenyl, 1-methylnaphthalene and 2-methylnaphthalene, has been assessed by the sulfobromophthalein (BSP) retention test. Liver function was moderately impaired after ingestion of 1.5 g/kg body weight of OM–2 and DOM, and of 2.0 g/kg THERMIP during the first two days after ingestion. HB–40 produced no effect. Among the pure compounds, *o*-terphenyl and 1-methylnaphthalene produced the strongest effects. In all cases normal liver function was restored after 3 to 5 days.

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THE ACUTE HEPATIC TOXICITY OF ORGANIC REACTOR COOLANTS*

1 — INTRODUCTION

Mixtures of high-boiling aromatic hydrocarbons, mainly oligophenyls and alkylated naphthalenes, are used as circulating liquids in the heat exchange systems of certain types of nuclear reactors. Under operating conditions these substances may be released from leaks of the piping system, and become airborne, forming very persistent aerosols, which are stabilized by electrostatic repulsion. After inhalation of these aerosols considerable amounts of the compounds may be resorbed by the body via the respiratory tract. Some components with comparatively low boiling point may be also present in the atmosphere at ambient temperature in the form of vapour in significant amounts. For the methylnaphthalenes, e.g., the saturation concentration at 25° is 0.6 g/m^3**. Absorption via the skin, which has been demonstrated for the methylnaphthalenes ([2]) and for diphenyl (1, p. 206) is another possible way of intake.

Since relatively little was known about the toxic properties of these compounds, a series of investigations was initiated. The present publication deals with the acute liver toxicity of the commercial mixtures OM–2, DOM, THERMIP and HB–40 and of their main components, diphenyl, *o*-terphenyl, *m*-terphenyl, 1-methylnaphthalene and 2-methylnaphthalene. The structural formulae of the principal compounds are given in Figure 1.

A special investigation on liver toxicity was justified by the assumption that, except for local irritation at the application site such as the lung or skin, the liver is supposed to be the primary site of the toxic action of these compounds. The toxicity of aromatic hydrocarbons is closely related to their metabolism ([3]). It is generally assumed that phenolic intermediates of the excretion pathways are responsible for most of the toxic effects. This is evident from the fact that alkylated aromatic hydrocarbons such as toluene or xylene are considerably less toxic than the corresponding non-substituted hydrocarbon, in this case benzene, since they are metabolized to relatively non-toxic aromatic acids, whereas relatively toxic phenols are formed from benzene. The "drug metabolizing enzymes", located in the endoplasmic reticulum of the liver cells, are responsible for the metabolic conversion of aromatic hydrocarbons to phenols. This first metabolic step is followed within the liver cells by a conjugation with glucuronic and/or sulfuric acid, resulting in the formation of water-soluble and less toxic glucuronides and sulfates, subsequently excreted via urine or bile. Consequently, the main toxic action of the phenolic intermediates can be expected to occur in the liver cells.

Histopathological changes in liver after acute doses of polyphenyls are reported in the literature. Moderately severe albuminous and fatty hepatocellular degenerations in rats and rabbits after oral application of diphenyl have been described by DEICHMANN *et al.* ([4]). Enlarged nuclei of rat liver cells were found by CORNISH *et al.* ([5]) after high doses of *o*-terphenyl, and ascribed to a regenerative activity of the liver.

Alterations of the metabolic activity of the liver as early as 6 hours after oral ingestion of terphenyls and methylnaphthalenes were detected by SCOPPA ([6]).

* Manuscript received on February 10, 1970.

** Extrapolation from vapour pressure data given by GERARDE (1, p. 217).

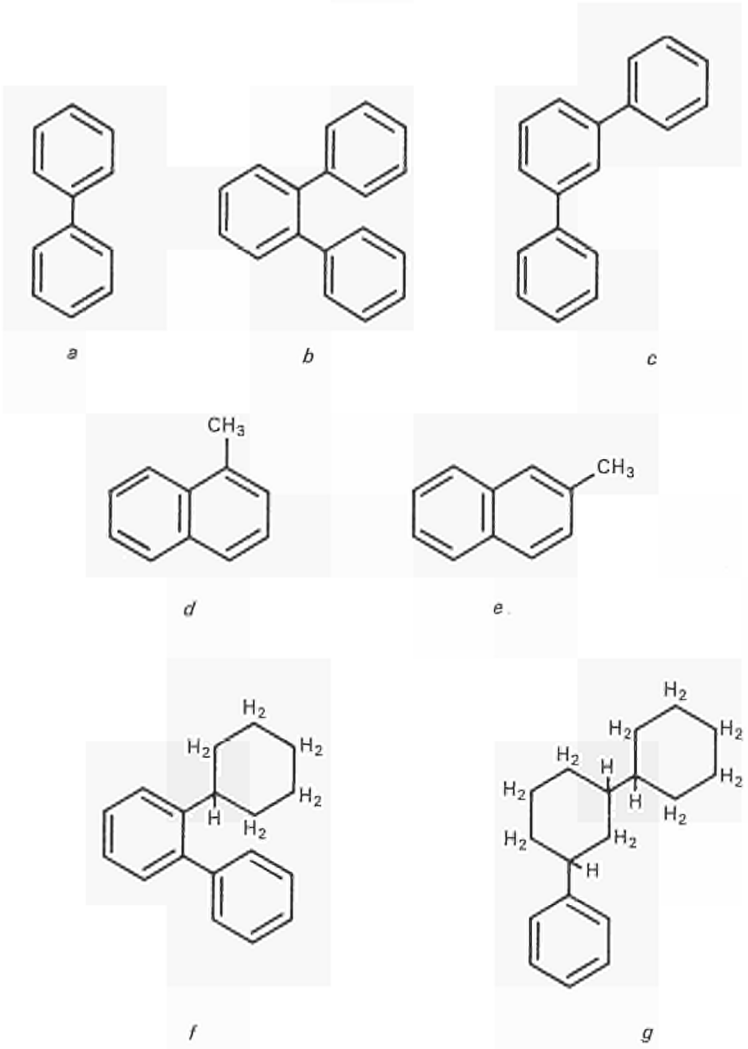

Fig. 1 — Main components of the organic reactor coolants OM–2, DOM, THERMIP and HB–40; *a*) diphenyl; *b*) *o*-terphenyl; *c*) *m*-terphenyl; *d*) 1-methylnaphthalene; *e*) 2-methylnaphthalene; *f*) and *g*) examples for partially hydrogenated terphenyls.

The sulfobromophthalein (BSP) retention test was used in our experiments as a measure of liver injury. This test is generally accepted to be sensitive, universal, and well correlated with morphological damage.

2 — MATERIALS AND METHODS

Male rats of the Sprague-Dawley strain, weighing 200-300 g, were maintained on a synthetic diet (A.L.A.L., Allevamento Lombardo Animali di Laboratorio, Milano), and water *ad libitum*.

OM–2 (Progil, France) is a mixture containing *o*-terphenyl (15-25 %), *m*-terphenyl (70-80 %), *p*-terphenyl (< 5 %), diphenyl (< 1 %).*

DOM (Monsanto, USA) is the eutectic mixture of diphenyl (26 %), *o*-terphenyl (58 %), *m*-terphenyl (15 %), *p*-terphenyl (1 %)*. OMB (Progil, France) is a similar mixture.

THERMIP (ESSO, France) is mainly a mixture of alkylated naphthalenes of varying composition. The batch used for our experiments contained naphthalene (2.5 %), 1-methylnaphthalene (27 %), 2-methylnaphthalene (54 %), dimethylnaphthalenes and related compounds (16.5 %)**.

HB–40 (Monsanto, USA), corresponding to OMP–H (Progil, France), is a complex mixture of terphenyls and partially hydrogenated terphenyls, in which about 40 % of the rings are saturated*. Examples of the constituents are given in Figure 1.

Coolants and pure compounds were given to the animals by stomach tube in an 1:1 *w/v* solution in olive oil, except for the less soluble terphenyls and OM–2, which were given in an 1:2 *w/v* suspension heated to about 40°C.

At various time intervals after application of the hydrocarbons the BSP retention test was carried out by the method we previously reported ([7,8]). The injection solution, containing 10.0 mg/ml BSP (phenoltetrabromophthalein sulfonic acid, tetrasodiumsalt) in isotonic saline, was prepared by dilution of a commercial preparation (Bromthalein ®, E. Merck AG., Darmstadt, 5 % aqueous solution). A dose of 20 mg/kg body weight was injected in the tail vein. Fifteen minutes after the injection, blood was sampled by cardiac puncture, and allowed to coagulate. Serum was isolated by centrifugation, and the BSP content was determined at 580 nm, using a UNICAM SP 600 spectrophotometer. The BSP concentration, expressed in μg/ml serum, obtained under these experimental conditions, was defined as standard retention ([7]). The standard retention of control animals was found to be 6.9 μg/ml serum. Groups of 5-7 rats were used for each point of the curves given in Figures 2-4. When large individual variations were found, the experiment was repeated with an increased number of animals.

3 — RESULTS

3.1 — Oligophenyls

3.1.1 — *Pure compounds*

Standard retention after ingestion of 1.5 g/kg of diphenyl, *o*-terphenyl and *m*-terphenyl is plotted in Figure 2 as a function of time. Diphenyl produces a slight, but significant ($P < 0.01$) increase of BSP retention on the first day, which returns to normal as early as 24 hours after ingestion. A steady increase of BSP retention can be observed after ingestion of *m*-terphenyl, reaching a maximum after 24 hours. Within 2 days liver function has returned to normal. The most severe effect is produced by *o*-terphenyl. BSP retention reaches a maximum after 16 hours and remains considerably high during the following day. The observed decrease during this period with a minimum at 20 h is at the limit of significance ($P = 0.05$), and seems not to be substantial. The biphasic peak is due to a few extremely high retentions influencing the means at 20 and 31 hours. After 5 days normal liver function can be observed.

* Specifications of the manufacturer.

** We thank Dr. B. Versino, Chemistry Department, EURATOM Ispra, for the gaschromatographic analysis.

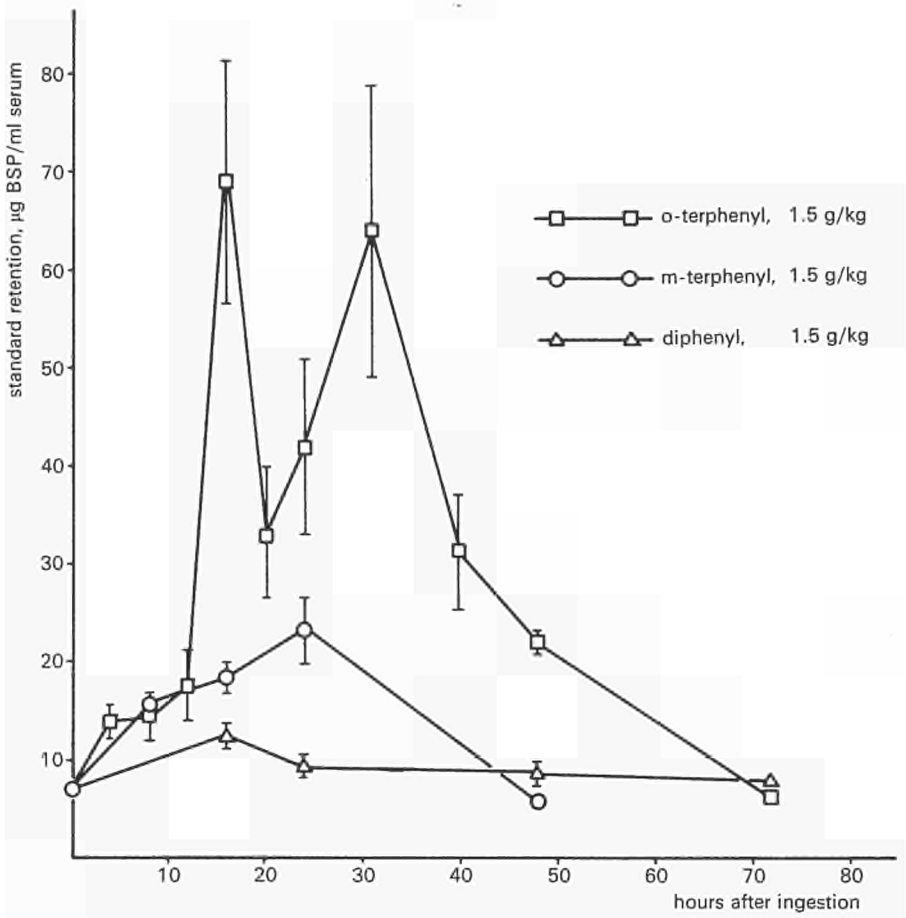

Fig. 2 — BSP standard retention in rat after ingestion of 1.5 g/kg diphenyl, *o*-terphenyl and *m*-terphenyl. Vertical bars indicate standard error.

3.1.2 — *Coolant mixtures*

Standard retention after ingestion of 1.5 g/kg of the technical products HB–40, DOM and OM–2 is reported in Figure 3. HB–40 does not produce a significant increase of BSP retention. Increased retentions with maxima at the end of the first day could be demonstrated for DOM, and even more so for OM–2. The effects can be roughly correlated with those of the pure compounds they contained and their concentrations in the mixtures. In both cases retentions return to normal values after 3 days.

3.2 — Alkylnaphthalenes

3.2.1 — *Pure compounds*

Standard retention after ingestion of 2.0 g/kg 1-methylnaphthalene and 2-methylnaphthalene is given in Figure 4. The most striking effect is produced by 1-methylnaphtalene.

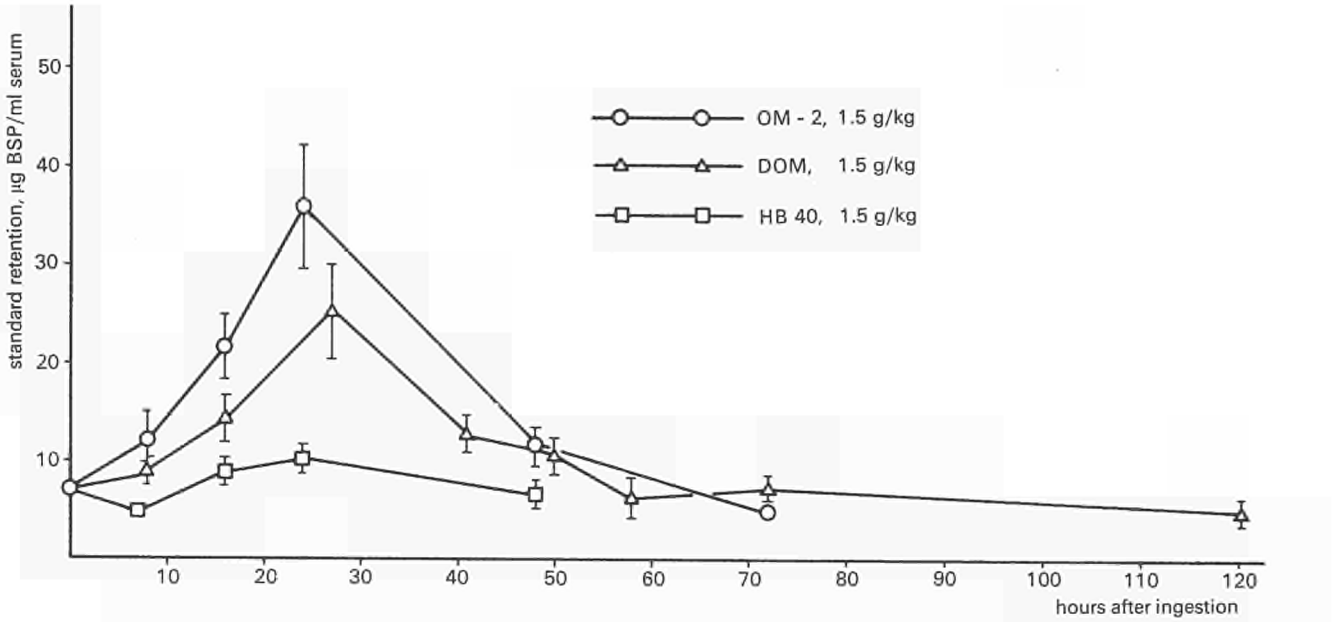

Fig. 3 — BSP standard retention in rat after ingestion of 1.5 g/kg HB 40, DOM and OM–2. Vertical bars indicate standard error.

Retention values show a steady increase to a remarkably high peak after 2 days, followed by a sharp decrease to almost normal retention after 3 days and completely normal retention after 5 days. 2-methylnaphthalene is less than half as active as the 1-isomer, and maximum retention occurs after 31 hours. Retention is normalized after 3 days.

3.2.2 — *Coolant mixtures*

Retention caused by THERMIP (Fig. 4) reaches a maximum at the end of the 2nd day and normal values after 3 days; the curve is approximately the sum of the curves of the main components taking into account their concentrations.

4 — DISCUSSION

From a toxicological point of view, the results lead to the following conclusions:

a) The organic reactor coolant OM–2, DOM and THERMIP* when ingested in comparatively high doses, give rise to a disturbance of liver function during the first 2 days after ingestion;

* The coolant ESSO SOLVENT 200, a mixture of methylnaphthalenes and about 10 % diphenyl, has not been investigated experimentally. Since the effect produced by diphenyl is rather small, this mixture with regard to hepatic toxicity can be assumed to behave like THERMIP.

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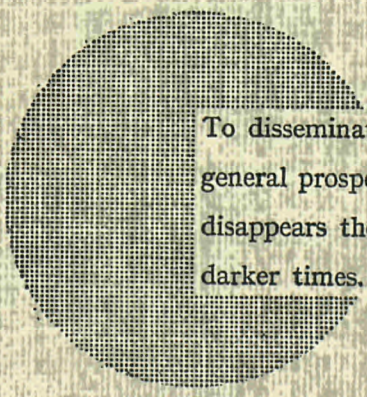

To disseminate knowledge is to disseminate prosperity — I mean general prosperity and not individual riches — and with prosperity disappears the greater part of the evil which is our heritage from darker times.

Alfred Nobel

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