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CHRONIC TOXICITY OF A MIXTURE OF ALKYLATED NAPHTALENES USED AS A NUCLEAR REACTOR COOLANT

by

H. OTT, K. GERBAULET and D. PIRRWITZ

1973

Joint Nuclear Research Centre
Ispra Establishment - Italy

Biology Division

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ABSTRACT

The effects of chronic administration to rat of THERMIP, a commercial mixture of alkylnaphthalenes, are reported. The animals were treated with a daily dose of about 0,3 mM/kg body weight for 5 months. At the end of the period histological examinations as well as a series of biochemical tests did not reveal pathological damage. Prolonged ingestion of alkylnaphthalenes gives rise to an induction of drug metabolizing enzymes in the liver, as made evident by the pentobarbital sleeping time test.

KEYWORDS

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EXCRETION
HEMATOLOGY
LIVER

CHRONIC TOXICITY OF A MIXTURE OF ALKYLATED NAPHTHALENES USED AS A NUCLEAR REATOR COOLANT [x])

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H. Ott, K. Gerbaulet and D. Pirrwitz

Introduction

Mixtures of alkylated naphthalenes are used as circulating liquids in the heat exchange systems of certain types of nuclear reactors. Since under operating conditions substantial amounts may be released and become airborne in the form of vapour and very persistent aerosols, they have to be considered as potential health risks for the operating personal. The most important aspect is a chronic or subacute intake which may occur via the respiratory tract. Vapour and sufficiently small droplets can be resorbed directly by the lung, bigger droplets are retained in the trachea, expectorated, and subsequently swallowed. Resorption via the skin is another possible way of intake, and has been demonstrated in rabbits (1).

Since relatively little is known about the toxic properties of these compounds, an extensive research program has been initiated. The present report is part of a series of publications on the toxicity of organic reactor coolants, and deals with the chronic toxicity of THERMIP, a commercial mixture of alkylated naphthalenes, in rat after oral intake. Results of treatment with THERMIP, in the form of aerosols, which may give rise, in addition to the general toxic effects, to local damage in the respiratory organs will be reported elsewhere (2).

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[x]) This publication is contribution n. 785 of Euratom Biology Division.

Materials and Methods

THERMIP (ESSO, France) is a mixture of alkylated naphthalenes in which the concentration of the different components may vary within certain limits. The batch used for our experiments contained naphthalene (2.5%), 1-methyl-naphthalene (27%), 2-methylnaphthalene (54%) and other alkylated naphthalenes and related compounds (16.5%)[x]. Ethylnaphthalene and several isomers of dimethylnaphthalene have been identified as components of the latter fraction. A more detailed quantitative analysis is extremely difficult due to the high number of possible structural isomers of polyalkylnaphthalenes.

48 male albino rats of the Sprague-Dawley strain (A.L.A.L., Allevamento Lombardo Animali di Laboratorio, Milano) were housed three in a cage, and maintained for 5 months on a synthetic diet (A.L.A.L., Milano) and water ad libitum. A paste was prepared with the powdered diet using an equal amount of water containing the suspended THERMIP. 36 animals received a diet containing 1 ml THERMIP/kg dry food during 5 days per week. 12 animals served as controls. Food intake at the beginning of the experiment was about 15 g dry diet per day and animal; it increased to about 20 g per day during the first 6 weeks, and remained constant for the rest of the period. The ingested dose increased from about 15 mg (0.1 mM) to about 20 mg (0.13 mM) per day. The total amount ingested was about 2 g per animal. The increase of body weight was registred at irregular intervals.

After a period of 114 days, 12 treated animals and 12 controls were subjected to the pentobarbital sleeping time test (3). Pentobarbital (40 mg/kg body weight) was administred by intravenous injection, which in adult rats proved more reliable than intraperitoneal application. The end of the sleeping time was defined by the presence of erecting reflex 3 times per minute.

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x) We thank Dr. B. Versino, Chemistry Division, EURATOM, Ispra, for the gaschromatographic analysis.

After 5 months, in some animals, liver function was assayed by the Sulfobromophthalein (BSP) retention test using the method elaborated by OTT and PIRRWITZ (4, 5). From the remaining animals blood samples were withdrawn by cardiac puncture for the assay of hemoglobin and bilirubin content, the determination of the respective activities of alkaline phosphatase (AP), glutamic oxalacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT), and for measuring the hematokrit value. Blood smears were prepared for the differential counts of blood components.

Hemoglobin was assayed by the cyanmethemoglobin method. Commercial test sets (Boehringer, Mannheim) were used for the determination of bilirubin content as well as for the determination of the respective activities of AP, GOT and GPT.

The sacrificed animals were dissected and examined for gross pathological damage. Liver, spleen, kidneys, heart and lungs were removed for weight determination. Thin sections of liver and kidney tissues were examined for histopathological alterations.

Results

Food uptake and increase of body weight

Food containing THERMIP was normally accepted by the animals. In fig. 1 increase of body weight is plotted against time. During the first 15 days there was no difference between treated animals and controls. Starting from the 15th day a slightly retarded weight increase of the treated animals can be observed. The difference between the two groups after 95 days is at the limit of significance ($P = 0.05$).

Pentobarbital sleeping time

The sleeping times measured after 114 days were 73.7 ± 3.3 min for the treated animals, and 96.4 ± 4.8 min for the controls (Ave. ± SE). The difference is highly significant ($P < 0.001$).

BSP retention test

BSP retention, 15 min after i.v. injection of 20 mg BSP per kg body weight, was 11.0 ± 0.9 µg/ml serum (Ave. ± SE of 8 animals) for the treated animals, and 13.2 ± 1.4 µg/ml serum (Ave. ± SE of 5 animals) for the controls. These values correspond to the normal retention found in rats of a mean body weight of 520 g (5). There is no significant difference between treated animals and controls.

Bilirubin and serum enzymes

The values for bilirubin content in the serum and for the activities of AP, GOT and GPT are given in table 1. No significant differences between treated animals and controls were evident.

Hematological examinations

The results of the hematological examinations are reported in table 2. No significant differences could be pointed out regarding the content of hemoglobin, the hematokrit value, and number and distribution pattern of leukocytes.

Gross pathological examination

The inner organs of the treated animals did not show any gross pathological damage. The relations between the weight of different organs and the total body weight given in table 3 do not show significant differences between treated animals and controls.

Histopathological examinations

Histopathological examination of liver and kidney tissues did not reveal pathological changes.

Discussion

An investigation of the toxic properties of foreign compounds has to take into account their general pathway in the body which is outlined by a list of 5 principal steps

a) absorption,
b) transport by the blood circuit,
c) metabolism in the liver,
d) transport of the metabolites by the blood circuit,
e) excretion of metabolites.

Methylnaphthalenes, like most lipid soluble aromatic hydrocarbons are readily absorbed by the body. This is shown by the physiological effects manifested already a few hours after ingestion, e.g. by the interference with the metabolism of pentobarbital (6) and by impairment of liver function (7).

The absorbed hydrocarbons are bound to serum proteins and carried to the liver. Within the liver cells they are transformed to highly toxic phenols by action of the drug metabolizing enzymes located at the endoplasmatic reticulum, and finally conjugated with glucuronic or sulfuric acid in order to make them soluble in water (8). The conjugated metabolites may be either directly excreted via the bile and gastro-intestinal tract or passed through circulation and kidney, and excreted via the urine.

Consequently, in this investigation special emphasis was put on possible damage to the liver, where during metabolism highly toxic phenolic intermediates are formed. This was also indicated in results from earlier work (7) which revealed a considerable, although completely reversible impairment of liver function after ingestion of high doses of THERMIP and of the pure methylnaphthalenes.

In order to allow valid conclusions for occupational medicine, the doses fed to the animals during this investigation, ca. 50 mg per kg body weight/day, 5 days per week, were considerably higher than those to be presumably ingested by man in the conditions of a chronic exposure at the working site.

Except for the pentobarbital sleeping time test, all biochemical tests performed revealed no damage or significant evidence for metabolic disturbances; pathological, histopathological and hematological examinations also showed no evidence of damage. Development of the animals was normal within statistical limits. BSP-test and bilirubin content of serum did not indicate impaired excretory function of the liver, and serum activities of the enzymes AP, GOT and GPT, which are sensitive indicators for necrotic processes in the liver (9), were not high in comparison with controls.

A detailed analysis is indicated for the results of the pentobarbital sleeping time test. The pentobarbital sleeping time, prolongation of which is considered to be a sensitive indicator of liver injury (3), was significantly lowered after treatment with THERMIP. The test is based on the fact that the narcotic pentobarbital is converted in the liver to inactive metabolites. If, in the case of liver injury, the metabolic rate is lowered, the serum concentration of the drug necessary for narcosis is maintained for a longer period, and sleeping time is extended. The contrary effect, as observed in our experiments, is also known; it is generally ascribed to an induction of the drug metabolizing enzymes in the liver, triggered by the charge with foreign compounds, and linked with a proliferation of the smooth endoplasmatic

reticulum in the liver cells (10). As a consequence, metabolism of pentobarbital is enhanced, and duration of narcosis is shortened. This effect has already been observed by SCOPPA after administration of a single dose of THERMIP (6).

Acknowledgements

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References

1) K. Gerbaulet and M. Roumengous, unpublished results.

2) K. Gerbaulet and H. Ott, The toxicity of organic coolants - Part II : Alkylnaphthalenes, EURATOM Report, to be published

3) T. Balazs and H.C. Grice, Toxicol. Appl. Pharmacol., 5, 387 (1963)

4) H. Ott and D.Pirrwitz, Clin. Chim. Acta, 22, 439 (1968)

5) H. Ott and D. Pirrwitz , Arch. Sci. biol., 53, 199 (1969)

6) P. Scoppa, Effects of Acute and Chronic Terphenyls and Methylnaphthalenes Administration on Hepatic Pentobarbital Metabolism in the Rat, EURATOM Report EUR 3254 e (1966)

7) H. Ott and D. Pirrwitz, The Acute Hepatic Toxicity of Organic Reactor Coolants, EURATOM Report EUR 4548 e (1970)

8) R.T. Williams, Detoxication Mechanism, 2nd ed., Chapman & Hall Ltd., London (1959)

9) T. Balazs, T.K. Murray, J.M. McLaughlan, H.C. Grice, Toxicol. Appl. Pharmacol., 3, 71 (1961)

10) H. Remmer, H.J. Merker, Ann. N.Y. Acad. Sci., 123, 79 (1965).

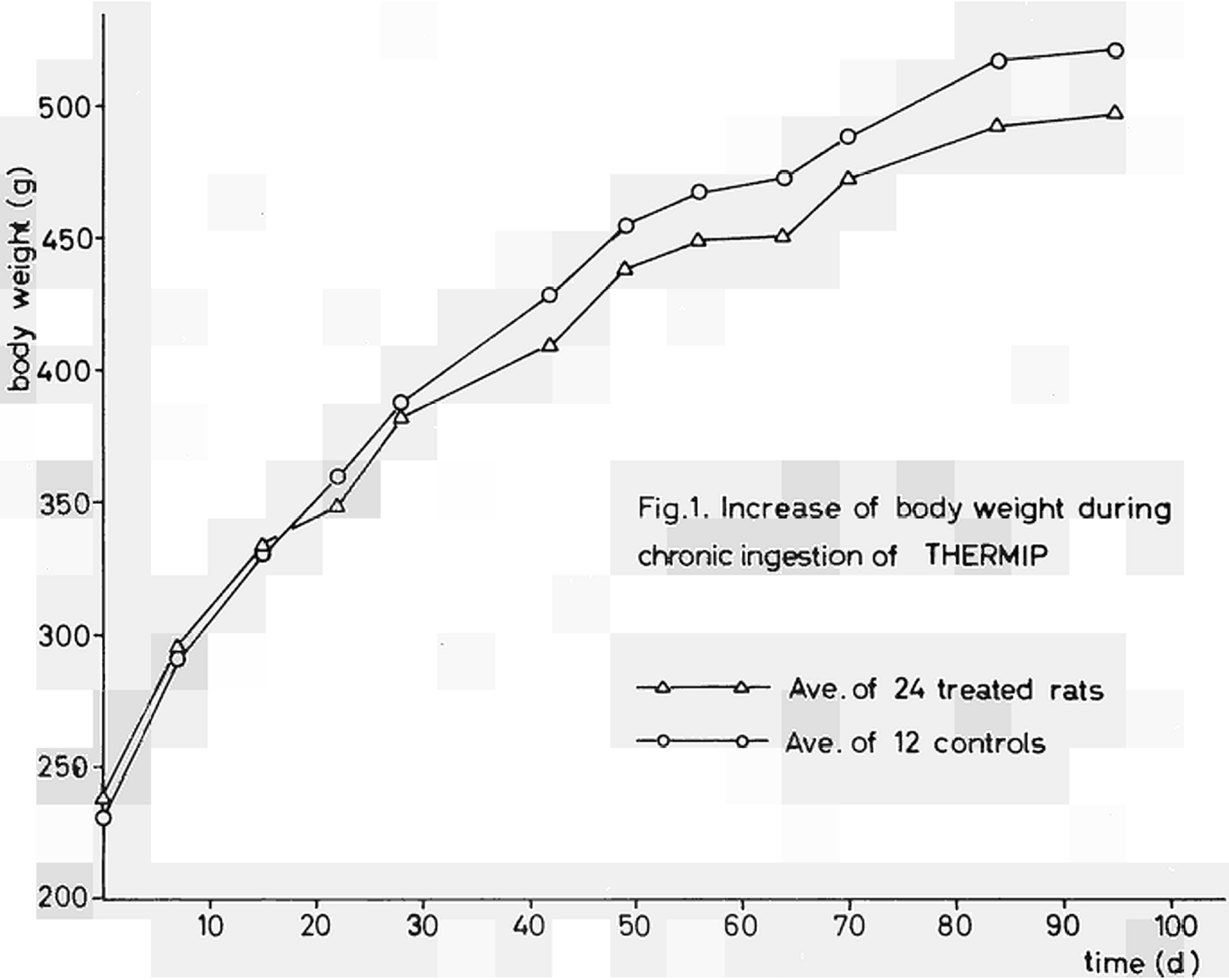

Fig.1. Increase of body weight during chronic ingestion of THERMIP

Table 1. Content of bilirubin and activities of some enzymes in rat serum after chronic ingestion of THERMIP

	bilirubin mg/100 ml	alkaline phosphatase (AP) mU/ml	glutamic oxalacetic transaminase (GOT) mU/ml	glutamic pyruvic transaminase (GPT) mU/ml
treated animals Ave. of 16 rats ± SE	0.11±0.02	103 ± 12	53.3 ± 3.5	9.9 ± 0.9
controls Ave. of 6 rats ± SE	0.05±0.01	160 ± 25	47.8 ± 3.9	9.4 ± 1.1

Table 2. Results of hematological examinations after chronic ingestion of THERMIP.

	hemoglobin g/100ml	hematokrit vol.%	number of leukocytes per mm^3	distribution of leukocytes (%)			
				lymphocytes	neutrophiles	monocytes	eosinophiles
treated animals Ave. of 19 rats ± SE	14.8 ± 0.2	46.8 ± 0.5	11100 ± 1060	65.3 ± 3.0	27.9 ± 2.4	5.1 ± 0.7	1.7 ± 0.9
controls Ave. of 10 rats ± SE	14.7 ± 0.3	47.9 ± 0.9	12060 ± 940	65.5 ± 3.0	27.7 ± 2.8	4.3 ± 1.2	2.5 ± 1.4

Table 3. Relation between the weight of different organs and the total body weight of rats after chronic ingestion with THERMIP.

	liver	spleen	kidneys	heart	lungs
treated animals $\frac{\text{organ weight} \cdot 100}{\text{body weight}}$ Ave. of 24 animals ± SE	336.0 ± 8.6	27.5 ± 1.0	69.2 ± 1.9	28.9 ± 0.7	40.0 ± 1.2
controls $\frac{\text{organ weight} \cdot 100}{\text{body weight}}$ Ave. of 12 animals ± SE	333.6 ± 8.9	27.3 ± 1.5	69.0 ± 1.4	26.7 ± 0.6	36.1 ± 1.0

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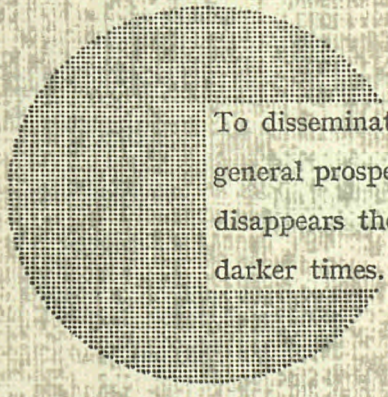

To disseminate knowledge is to disseminate prosperity — I mean general prosperity and not individual riches — and with prosperity disappears the greater part of the evil which is our heritage from darker times.

Alfred Nobel

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