International Journal of Occupational Medicine and Environmental Health, Vol. 11, No 4, 305–317, 1998

ORIGINAL PAPERS

DISPOSITION AND METABOLISM OF 1,2-DIMETHYLNAPHTHALENE IN RATS

ANNA KILANOWICZ and ANDRZEJ SAPOTA

Department of Toxicological Chemistry
Medical University
Łódź, Poland

Key words: 1,2-Dimethylnaphthalene-[ring-U-^{3}H], Distribution, Excretion, Metabolism, Rats

Abstract. The aim of this study was to investigate the distribution, excretion and metabolism of 1,2-dimethylnaphthalene-[ring-U-^{3}H] in rats.

The experiments were performed on 54 male outbred IMP:Wist rats with body weight of 200 g ± 20%. The compound was given i.p. in olive oil in a single dose of 28 mg/kg (about 6.2 MBq per animal). ^{3}H radioactivity was traced in selected organs and tissues, blood, urine and faeces, 1–72 h following the administration. The main metabolites were isolated from urine and identified by the GC-MS method.

Faeces and urine proved to be the main route of tritium elimination. Over 93% of the given compound was excreted during the first 72 h. Maximum level of tritium in plasma was observed during the 4th h after the compound administration. The accretion of ^{3}H proceeded with kinetic constant of 0.7 h, followed by monophasic decline with the half-life of about 19h. In organs and tissue, the highest concentration during the first hours after administration were detected in the fat, adrenals, liver, spleen and kidneys. Then gradual decline of tritium was noticed in all examined tissues. The following urinary metabolites were identified: 1. 1,2-dimethylthionaphthalene, 2. 1,2-dimethylnaphthol, 3. 1-methylnaphthalene-2-methanol, 4. 1-methyl-2-naphthoic acid and 5. 1,2-dimethylmethylthionaphthalene.

In conclusion, 1,2-dimethylnaphthalene has a relatively rapid turnover rate in the rat organism and does not form deposits in the tissue. The metabolism encompasses ring hydroxylation and glutathione conjugation leading to thionaphthol and oxygenation, and then to naphthoic acid.

INTRODUCTION

In recent years there has been a noticeable growth in demand for products obtained from crude oil. Organic solvents which have become indispensable, in different branches of industry, belong to such compounds.

Address reprint requests to Assoc. Prof. A. Sapota, Department of Toxicological Chemistry, Medical University, Muszyńskiego 1, 90-151 Łódź, Poland.

Most of the solvents in use contain, among other compounds, methyl and ethyl derivatives of naphtalene. These compounds are also present in petrol (up to 2%) and in engine oil (up to 3%) (3,7,29).

Naphthalene is a major feed stock for the dye industry and is also used as an ingredient in paints and lacquers (21). The mononitro-, and monomethylnaphthalenes are produced commercially in smaller quantities, but are also important industrial intermediates (25). Methylnaphthalene was widely used industrially as a solvent for pesticides as a mordant carrier (18).

Polymethylnaphthalenes in general, and 1,2-dimethylnaphthalene in particular, have been used as solvents for fungicides (12) and as adjuncts in the formulations for the application of organophosphorous pesticides (27). A wide use of naphtalene derivatives has contributed to their emission into the environment. The presence of numerous derivatives of naphtalene in water and sea creatures has been described by a number of authors (17,19,23), while the data concerning the toxicity of naphtalene derivatives and their effect on humans, as well as on experimental animals are scarce or incomplete.

The immediate toxic effects of naphthalene are reasonably well known, but biological effects of most derivatives have not as yet been extensively studied (21). The experiments reported were intended to obtain information on relative toxicity of monomethylnaphtalenes, and to find out whether possible exposure hazards to humans might exist (21).

The experiments on rats and mice conducted by various authors showed that 2-methylnaphtalene and naphtalene produced a specific pulmonary lesion in mammalian species, namely necrosis of the nonciliated bronchiolar lining (Clara) cell (2,8,9,10,15,22,27,28). Bronchiolar Clara cells are considered to be a principal target in the mammalian lungs for xenobiotic pulmonary toxicants (20).

Cytochrome P-450-mediated metabolism of naphthalene results in the dose--dependent formation of reactive metabolites which deplete glutathione and become bound covalently to tissue macromolecules *in vivo* (28).

These data combined with the observation that high doses of naphthalene result in nearly complete elimination of pulmonary reduced glutathione suggest that reactive metabolites of naphthalene reach nearly all glutathione-containing cells in the lung. Thus it is most likely that reactive metabolites of naphthalene are formed in the liver and being sufficiently stable circulate to the lung (28).

Metabolism of dimethylnaphtalenes has not been comprehensively examined in mammals, although it is suspected that they might alter metabolism of carcinogenes (benzo[a]pyrene and dimethylbenzoanthracene) by interfering with their metabolism (6,16,26).

The aim of this study was to investigate the disposition of 1,2-dimethylnaphthalene-[ring-U-^{3}H] in the rat after a single, i.p. administration of this compounds and identification of the main metabolites excreted with urine.

MATERIALS AND METHODS

Animals. Adult male outbred IMP: Wist rats (Rattus) of 180–220 g body weight were obtained from the breeding colony of the Nofer Institute of Occupational Medicine in Łódź. The animals were supplied at least one week before the experiment and were fed a standard pelletized Murigran diet (Agropol, Motycz, Poland) with free access to water.

Chemicals. 1,2-dimethylnaphthalene-[ring-U-^{3}H] (1,2-DMN) with specific activity of about 900 MBq/g was purchased from the Department of Radiochemistry, Insitute of Radiation Technique, Technical University of Łódź. Cold 1,2-DMN of analytical grade, (Aldrich, England) was used for dilution of radioactive samples, as well as for the identification of urinary metabolites. The liquid scintillator Aquasol 2 was obtained from Du Pont; perchloric acid and perhydrol were purchased from Merck.

Animal treatment. The experiments were performed on 54 rats. The animals were put individually in glass metabolism cages (Simax, the former Czechoslovakia) and acclimatized for 48 h. Subsequently, animals were administered intraperitoneally 1,2-DMN dissolved in olive oil, in a single dose of 28 mg/kg of body weight (about 6.2 MBq per animal). Immediately after administration, the rats were placed in individual metabolism cages which enabled the collection of separate samples of urine and faeces. Blood samples (30 μl) were collected from the tail vein using calibrated, heparinized capillaries. Rats were decapitated under light-ether narcosis at appropriate time intervals and examined organs and tissues were removed for determination of radioactivity.

In all the experiments, the Polish Law on the protection of animals was followed (5).

Sampling of biological material and measurements of ^{3}H-radioactivity

Red blood cells, faeces (10% water homogenates) and 20% tissue organs homogenates were digested according to Mahin and Lofberg (14).

Urine samples, diluted with water to the volume of 50 ml and plasma samples were subjected to direct measurements. Radioactivity of samples, placed in glass scintillation vials with 15 ml of scintillator was measured using a LKB-Wallac 1209 Rackbeta liquid scintillation counter, according to the external standard method.

Isolation and identification of urinary metabolites

Urine samples (2.5 ml) collected during the first 24 h were acidified (pH = 1) and extracted with ethyl ether. Ether extract samples (1 μl) were injected into the GC-MS system. Mass detector worked at the SCAN mode at mass range 15–350 U. The GC-MS system from Hewlett Packard consisted of: (a) Gas Chromatograph HP 5890 equipped with capillary column HP-5; length 50 m; (b) Mass selective detector HP 5970 MSD; and (c) MS Chem. Station 59970c. Working parameters were as follows: injector temperature – 250°C; programmed temperature of the oven – initial temperature of 50°C immediately followed by a linear rise of 5°C/min and then of 10°C/min; separation time – 40 min. Helium was used as a carrier gas.

The identification of metabolites was based on the comparison of examined sample mass spectra with mass spectra contained in the computer data base. In the absence of the latter, mass spectrum analysis was performed. Where peaks differing in retention time showed essentially identical mass spectra, the presence of isomers was assumed. Due to lack of synthetic standard substances we were not able to identify the isomeric structure of metabolites.

The amounts (in %) of the identified metabolites were examined by comparing peak surfaces presented in chromatographic diagram.

Statistical analysis of the data was carried out using Sigma Stat. 1.0 for DOS. Sigma Plot 3.0 (Jandel Scientific) was employed to calculate kinetics of ^3H disposition in blood.

RESULTS

The kinetics of absorption and excretion

The excretion of ^3H following a single i.p. administration of 1,2-DMN at a dose of 28 mg/kg is presented in Table 1. Faeces and urine proved to be the main route of elimination. Over 93% of the compound was excreted during the first 72 hours.

The kinetics of ^3H accumulation and decline of its radioactivity in the blood plasma and RBC during 72 h following 1,2-DMN - administration at a dose of 28 mg/kg is presented in Figs. 1 and 2.

Table 1. Excretion of ^3H after a single, i.p. administration of 1,2-DMN-[ring-U-^3H] at a dose of 28 mg/kg in rats

Medium	% of the given dose			
	0–24 h	24–48 h	48–72 h	Total after 72 h
Urine	30.25±11.67	7.75±1.87	3.2 ±1.93	41.20
Faeces	34.47±11.99	11.52±2.40	6.31±2.87	52.30

The values are presented as mean ±SEM for 6 rats.

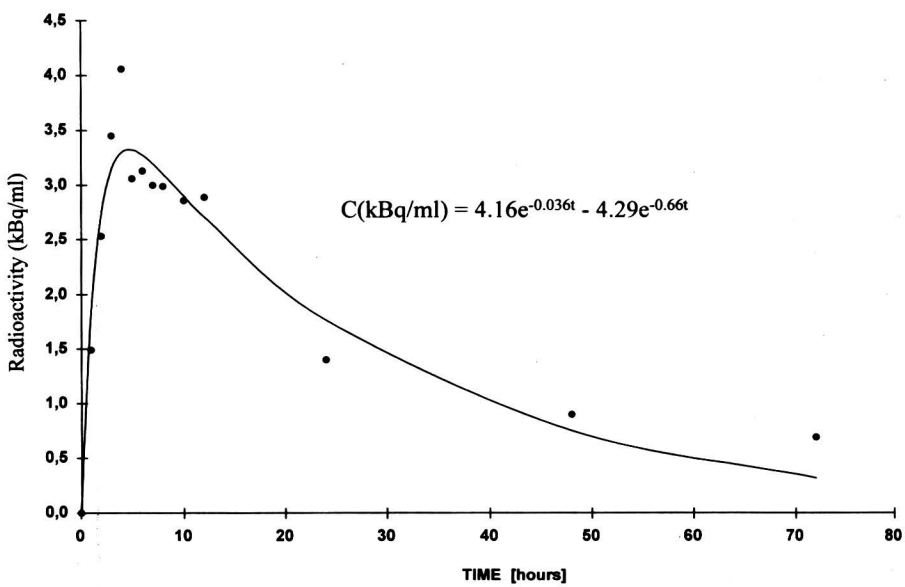


Fig. 1. The kinetics accretion and decline of ^3H radioactivity in plasma following a single, i.p. administration of 1,2-dimethylnaphthalene-[ring-U-^3H] at a dose of 28 mg/kg in rats.

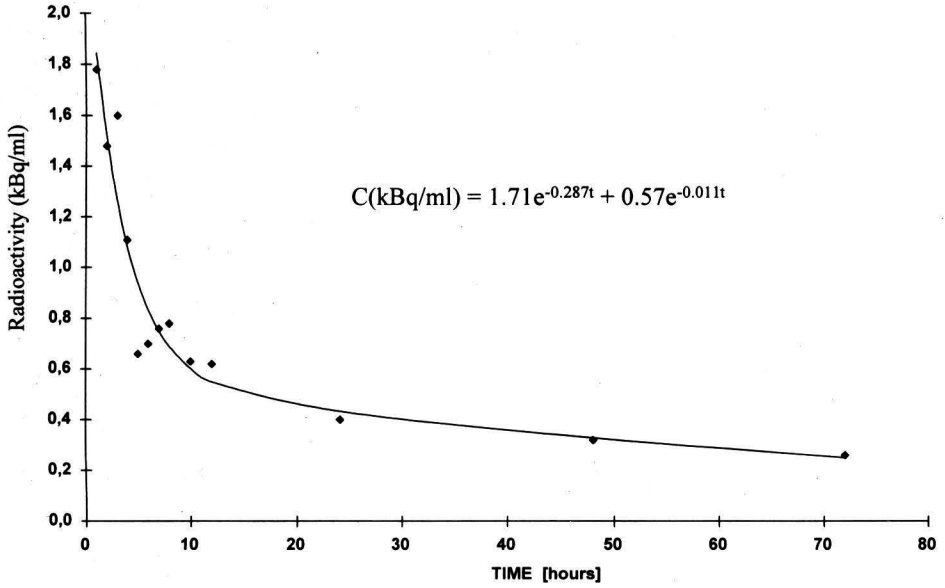


Fig. 2. The kinetics of ^{3}H distribution in RBC following a single, i.p. administration of 1,2-dimethylnaphthalene-[ring-U-^{3}H] at a dose of 28 mg/kg in rats.

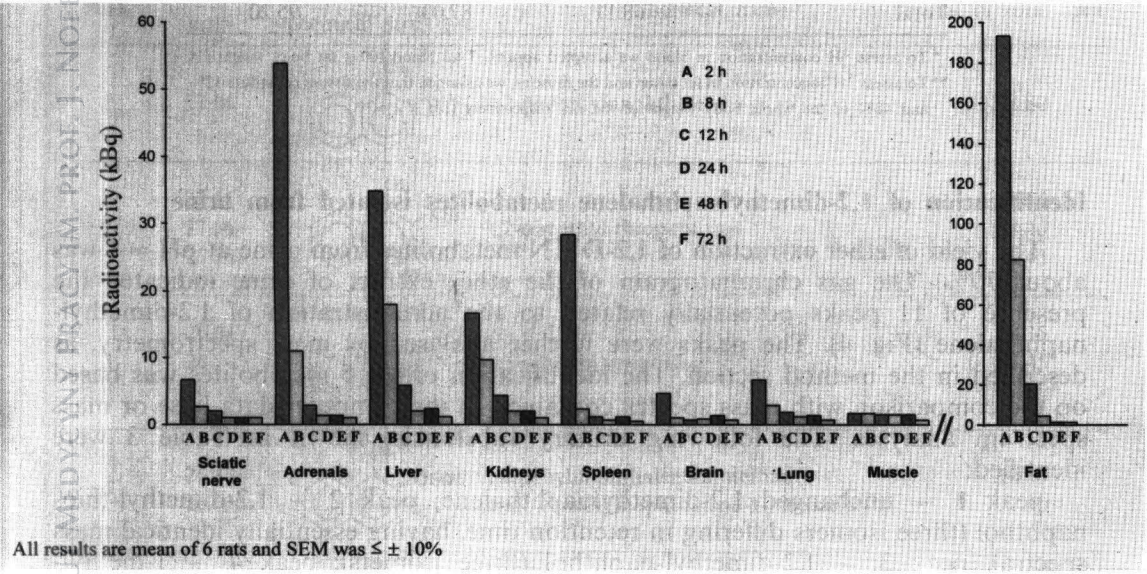


Fig. 3. Tissue and organs distribution of tritium in rat after a single, i.p. administration of 1,2-dimethylnaphthalene-[ring-U-^{3}H] at a dose of 28 mg/kg.

Maximum level of tritium in plasma was observed during the 4th h after administration of the compound. The accretion of ^{3}H in plasma proceeded with kinetic constant of 0.66 h, followed by monophasic decline of ^{3}H with the half-life

of about 19 h (Fig. 1), whereas ^3H decline in erythrocytes was biphasic, according to the equation presented in Fig. 2. Half-lives for phases I and II were 2.4 and 33 h, respectively.

The specific activity of ^3H in the rat organs and tissues after administration of 1,2-DMN at a dose of 28 mg/kg is presented in Fig. 3. Two hours after administration the highest concentrations were detected in the fat, adrenals, liver, spleen and kidneys. Then gradual decline of tritium was noticed in all examined tissues. Table 2 presents the total amount of tritium accumulated in organs and tissues, together with the amounts excreted. After 72 h, faeces and urine played a decisive role in the total balance.

Table 2. Total balance of tritium following a single, i.p. administration of 1,2-DMN-[ring-U-^3H] at a dose of 28 mg/kg in rats

Medium	Time following the administration (% of the dose)		
	0–24 h	0–48 h	0–72 h
Urine	30.25	38.0	41.20
Faeces	34.47	45.99	52.30
RBC + plasma*	0.31	0.26	0.23
Fat tissue**	0.34	0.09	0.07
Muscles**	2.32	2.29	1.17
Remaining tissues	0.11	0.13	0.06
Total	68.11	87.03	95.20

* To assess ^3H concentration in blood we accepted approx. 7 ml blood/100 g rat body weight (1),
** To assess ^3H concentration in fat tissue and the muscles, we assumed that the tissues constitute 12% and 40% of the whole body weight of the rat, respectively (13).

Identification of 1,2-dimethylnaphthalene metabolites isolated from urine

The yield of ether extraction of 1,2-DMN metabolites from urine at pH = 1 was about 70%. The gas chromatogram of the ether extract of urine indicated the presence of 11 peaks potentially related to the administration of 1,2-dimethylnaphthalene (Fig. 4). The peaks were further analysed by mass spectrometry, as described in the method section. The identification of the 5 metabolites was based on the comparison with mass spectra contained in the computer data base or mass spectrum analyses. The following urinary substances shown in Table 3 were identified:

peak 1 – unchanged 1,2-dimethylnaphthalene; peak 2 – 1,2-dimethyl-thionaphthol (three isomers differing in retention time, having essentially identical mass spectra); peak 3 – 1,2-dimethyl-naphthol (three isomers); peak 4 – 1-methylnaphthalene-2-methanol, and 2-methylnaphthalene-1-methanol; peak 5 – 1-methyl--2-naphthoic acid; and peak 6 – 1,2-dimethyl-methylthionaphthalene. Examples of typical mass spectra of the examined substances and their standards are presented in Figs. 5–8.

Basing on the comparison of the peak area in the chromatographic diagramme (Fig. 4) the relative contribution of individual metabolites was calculated (total area = 100%). Table 3 indicates that the main urinary metabolites resulting

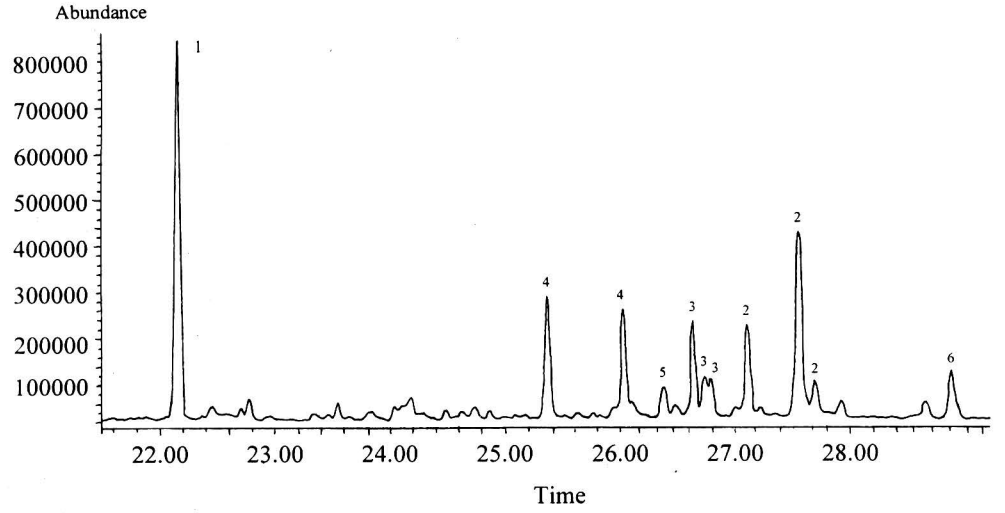
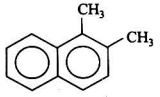
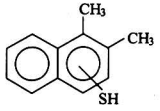
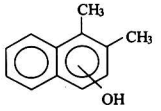
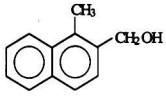
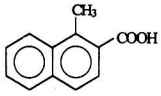
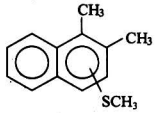


Fig. 4. A typical gas chromatogram of the ether extract of urine following administration of 1,2-dimethylnaphthalene.

Table 3. Contribution of identified urinary substances calculated by peak area of chromatogram (Fig. 4)

Peak	Retention time	Suggested chemical strukture	Chemical name	% of total area
1	22.16		1,2-dimethylnaphthalene	28.80
2	27.11 27.56 27.70		1,2-dimethyl-thionaphthol	7.85 18.40 3.45
3	26.64 26.74 26.79		1,2-dimethyl-naphthol	8.35 3.45 275
4	25.37 26.03		1-methylnaphthalene-2-methanol and 2-methylnaphthalene-1-methanol	10.80 7.90
5	26.39		1-methyl-2-naphthoic acid	2.80
6	28.89 29.38		1,2-dimethyl-methylthionaphthalene	5.45

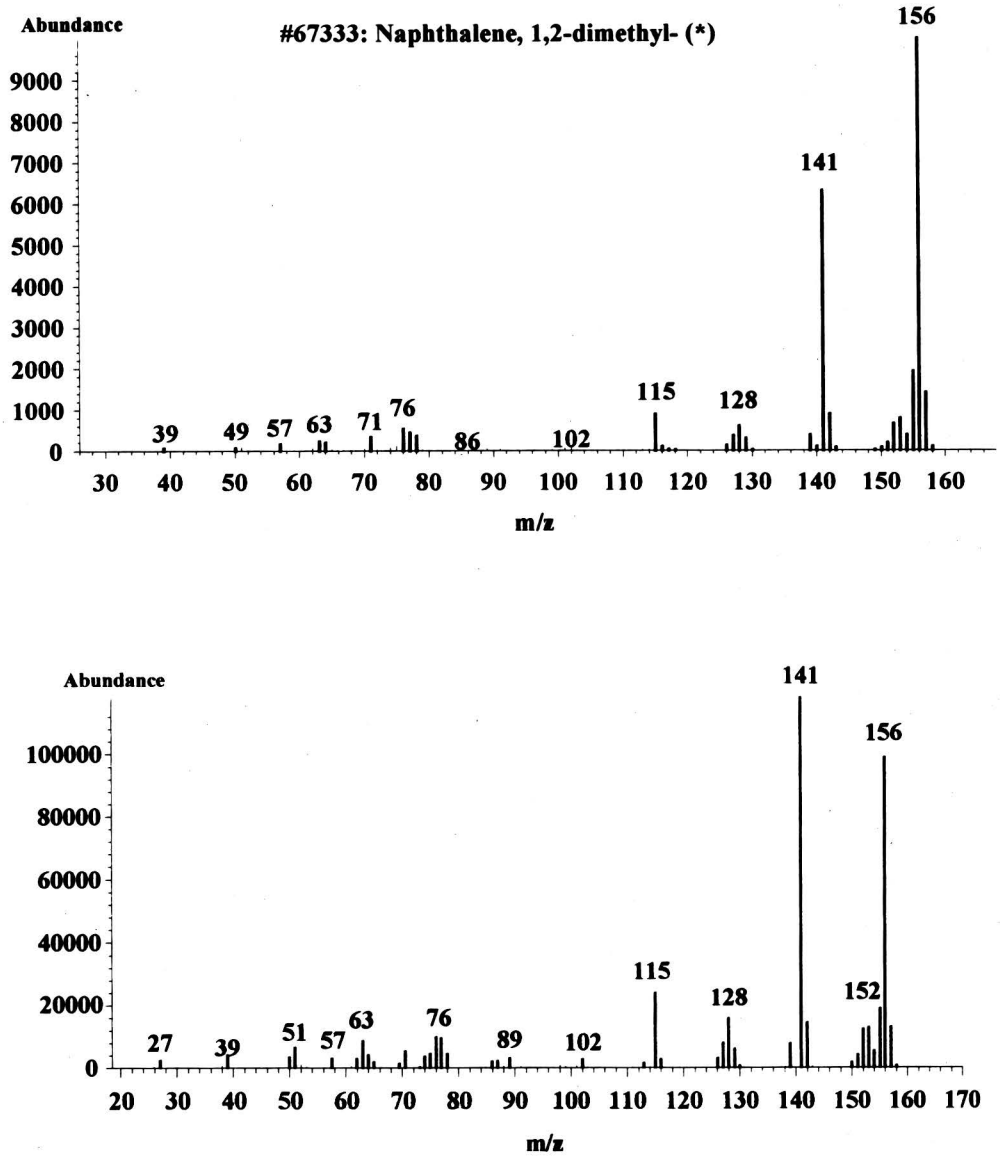


Fig. 5. A typical mass spectra of the examined substances (peak 1) and their standard.

from administration of 1,2-DMN are three isomers of 1,2-dimethyl-thionaphthols (almost 30%) and unchanged 1,2-DMN (almost 29% of the total amount excreted with urine).

Tentative pathways of 1,2-DMN biotransformation (Fig. 9) comprise: 1. ring hydroxylation (direct or through expoxide) resulting in dimethylnaphthols, 2. glutathione conjugation resulting in 1,2-dimethylthionaphthols and 3. alkyl oxidation resulting in 1-methylnaphthalene-2-methanol and 1-methyl-2-naphthoic acid.

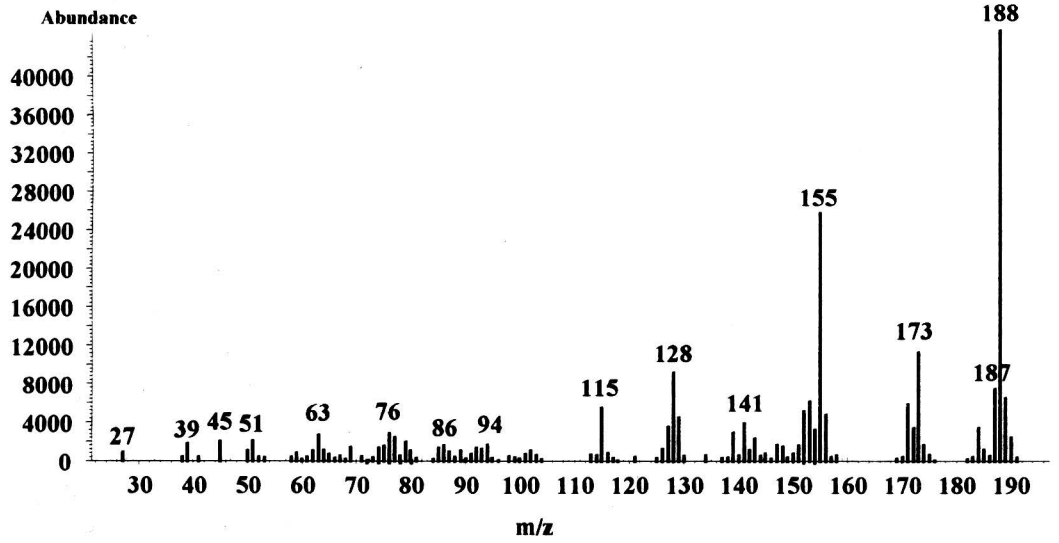


Fig. 6. A typical mass spectra of the examined substances (peak 2). The postulated formula: $C_{12}H_{12}S$. The overall value of double bond equivalent (DBE) equals 7. Parent ion M = 188 u. A value of isotope ion [M + 2] = 190 u amounts of about 5% parent ion value which suggests the presence of sulphur atom in the molecule. Postulated fragmentation: 1. [M − 15] → 173 u (demethylation), 2. [33] → 155 (detachment of [SH]* group), 3. Further fragmentation with rearrangement of formed ions.

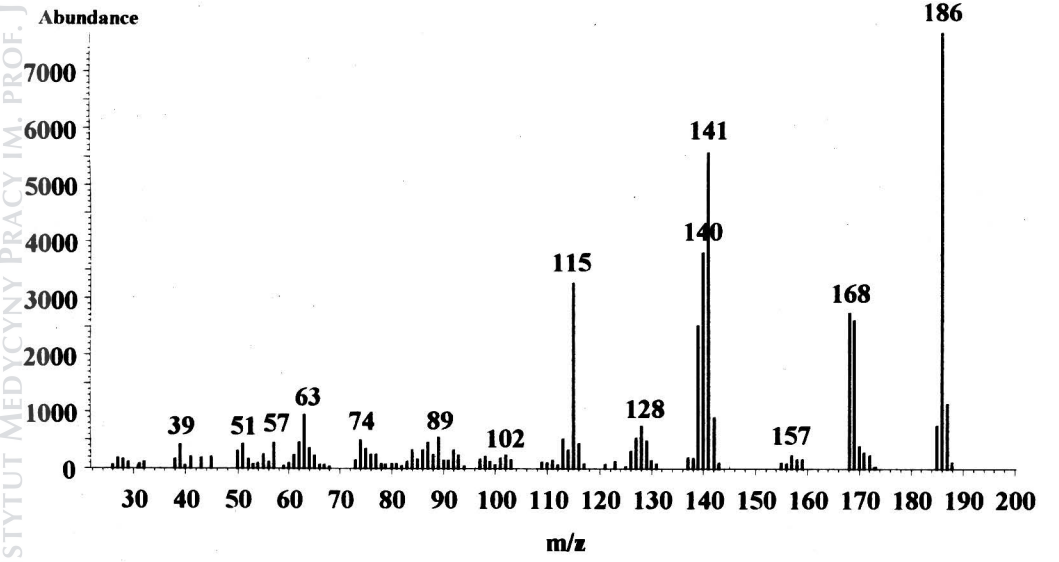


Fig. 7. A typical mass spectra of the examined substances (peak 5). The postulated formula: $C_{12}H_{10}O_2$. DBE = 8. Parent ion M = 186 u. Configuration of ions (M and M + 2) does not suggest the presence of heteroatoms (S,Br,Cl) in the molecule. Postulated fragmentation: 1. [M − 18] → 168 u (dehydration), 2. [M − 45] → 141 u (decarboxylation), 3. Further fragmentation with rearrangement of formed ions.

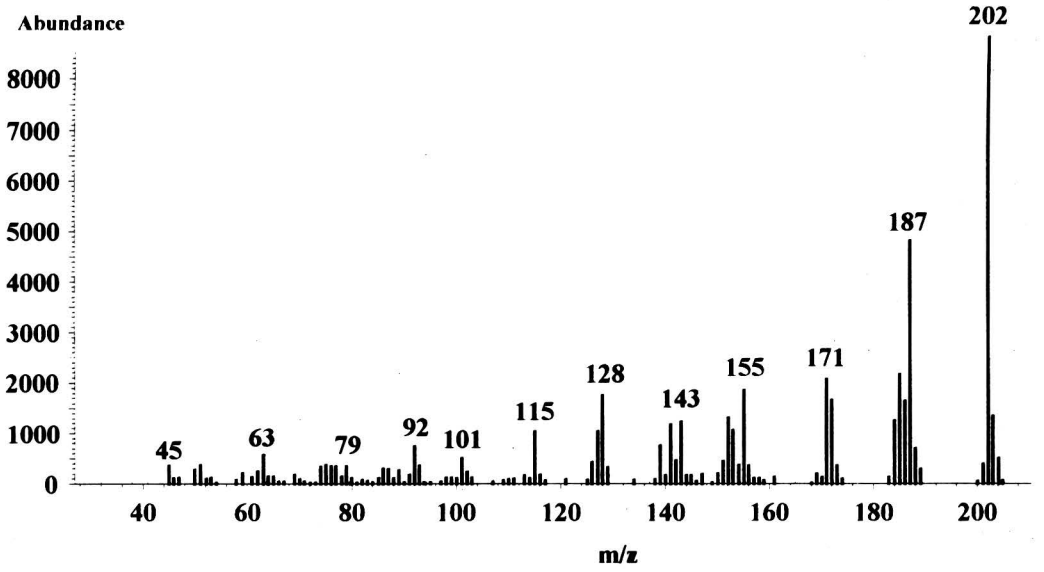


Fig. 8. A typical mass spectra of the examined substances (peak 6). Postulated formula: $C_{13}H_{14}S$. BDE = 7. Parent ion M = 202 u. The value of isotope ions [M + 2] = 204 u amounts of about 5% parent ion value which suggest the presence of sulphur atom in the molecule. Postulated fragmentation: 1. [M − 15] → 187 u (demethylation), 2. [187 − 32] → 155 u (desulphurization), 3. Further fragmentation with rearrangement of formed ions.

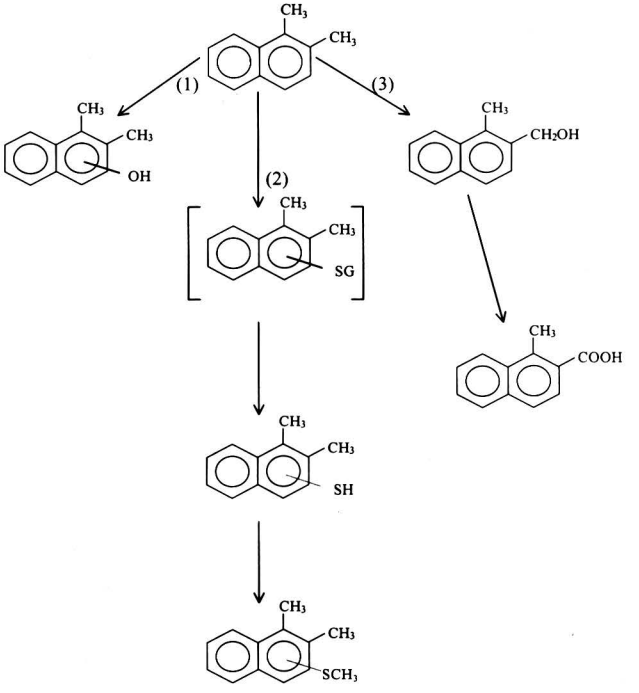


Fig. 9. Tentative metabolic pathways of 1,2-dimethylnaphthalene in rats. Hypothetical intermediates in brackets.

DISCUSSION

This report presents data on the distribution and excretion of 1,2-DMN obtained by radiotracer studies in rats, as well as by metabolic studies using the GC-MS technique.

1,2-DMN administered intraperitoneally at a dose of 28 mg/kg was absorbed rather quickly from the peritoneal cavity into the blood and then distributed evenly in tissues. An analysis of ^{3}H distribution in the blood and tissues, the rate of its decline and elimination with urine and faeces, revealed that the tritium did not form deposits in tissues and was shortly eliminated from the organism. The estimated half-lives in blood and a low total amount of ^{3}H in tissues 24 h following 1,2-DMN administration confirm the above finding.

From the yield of ether excretion with urine, the data may indicate the presence of 'free' unconjugated compounds or labile conjugates, prior to hydrolysis in urine pH = 1. Apart from the unchanged substance (1,2-DMN) the rat urine contains metabolites obtained in three different pathways. Firstly, 1,2-DMN may undergo ring hydroxylation yielding 1,2-dimethylnaphthols (three isomers). It seems that such reaction could proceede either directly, or possibly through epoxide. In the scheme (Fig. 9) the former possibility was accepted. The second pathway reflects the process leading to 1,2-dimethylthionaphthols. It is commonly known that such process involves reduced glutathione capable of enzymatically forming conjugates with organic xenobiotics. The mechanism by which such a conjugate is further split releasing naphthylthiophenol involves gradual enzymatic hydrolysis leading to cystene-S-conjugate which is further enzymatically converted, through release of pyruvate and ammonia, into the respective thionaphthol. Finally, methabolites having accessible sulphhydryl groups may undergo methylation, yielding 1,2-dimethyl-methylthionaphthalene. The third pathway reflects the process of oxygenation of alkyl derivatives of aromatic compounds to carboxylic acids (methylnaphthoic acid).

In the study carried out by Kaubisch et al. (10), the *in vitro* metabolism of 1,2-DMN using guinea pig microsomes reportedly gave rise to methylnaphthoic acid, dimethylnaphthol, and dimethylnaphthalene dihydrodiol. The latter metabolite was not identified in our study.

Our quantitative evaluation shows that apart from almost 30% being excreted as parent compound, about 36% of the metabolism products in urine contain oxygen and less than 35% contain sulfur. The presence of sulfur containing metabolite is relevant bearing in mind that 1,2-DMN in high dose causes depletion of hepatic GSH levels in the liver and lung.

It should be noticed that the total amount of metabolites excreted with urine constituted approximately 40% of the administered dose, whereas more than 50% of metabolites were excreted in faeces. Since the compound was administered intraperitoneally, it is obvious that 50% of the given compound were excreted with bile. The metabolites eliminated in faeces were not identified in this study.

In conclusion, 1,2-DMN has a relatively rapid turnover rate in the rat organism and does not form deposits in the tissue. The metabolism encompasses hydroxylation, and glutathione conjugation leading to thionapahthol and oxygenation, and then to naphthoic acid.

REFERENCES

1. Brij M, Mitrnka H, Rawnsley M. Clinical Biochemical and Hematological Reference Values in Normal Experimental Animals. Masson Publishing Inc., New York 1977.
2. Buckpitt A, Warren D. Evidence for hepatic formation, export and covalent binding of reactive naphthalene metabolites in extrahepatic tissues *in vivo*. J Pharm Exp Therapeutic 225: 1, 8–16, 1982.
3. Clark C, Henderson T, Royer R, Brooks A, McClellan R, Marshall W, Naman TT. Mutagenicity of diesel exhaust particle extracts: Influence of fuel composition in two diesel engines. Fundamental Appl Toxicol 2: 38–43, 1982.
4. Commandeur JNM, Stijntjes GJ, Vermeulen NPE. Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. Pharmacol Rev 47: 271–330, 1995.
5. Journal of Laws 111: 3445–3453, 1997.
6. Franklin R. Methylnaphtalenes. In: Ethel Browning's Toxicity and Metabolism of Industrial Solvents. 2nd edition. Vol. 1. Hydrocarbons. R. Snyder, ed. Elsevier Science Publishers B.V. 1987.
7. Fruchter J, Land J, Peterson M, Ryan P, Turner M. High precision trace element and organic constituent analysis of oil shale and solvent refined coal materials. In: Analytical Chemistry of Liquid Sources, Tar Sands, Oil Shale, Coal and Petroleum, Advances in Chemistry Series No. 170. PC Uden, S Siggia, HB Jensen, eds. American Chemical Society, Washington, D.C., 1978.
8. Griffin KA, Johnson CB, Breger RK, Franklin RB. Effects of inducers and inhibitors of cytochrome P-450-linked monoxygenases on the toxicity, *in vitro* metabolism and *in vivo* irreversible binding of 2-methylnaphthalene in mice. J Pharm Exp Ther 221: 517–524, 1982.
9. Griffin KA, Johnson CB, Breger RK, Franklin RB. Pulmonary toxicity, hepatic and extrahepatic metabolism of 2-methylnaphthalene in mice. Toxicol Appl Pharmacol 61: 185–196, 1981.
10. Griffin KA, Johnson CB, Breger RK, Franklin RB. Pulmonary toxicity of 2-methyl-naphthalene: lack of a relationship between toxicity, dihydrodiol formation and irreversible binding to cellular macromolecules in DBA/2J mice. Toxicology 26: 213–230, 1983.
11. Kaubisch N, Daly JW, Jerina DM. Arene oxides as intermediates in the oxidative metabolism of aromatic compounds. Isomerization of methyl-substituted arene oxides. Biochemistry 11: 3080–3088, 1972.
12. Konja T, Kitogaki T, Konagai Y. Fungicidal Compositions Containing 2,4,6-Tetra-Chloroisophthalonitrile and Polymethylnaphthalene. Japanese Patent No 7701022 to Kumiai Chemical Industry Co. Ltd 1977.
13. Krzymowski T. Physiology of animals. PWRL, Warsaw 1973 (in Polish).
14. Mahin DT, Lofberg RT. A simplified method of samples preparation for determination of tritium, carbon-14 or sulfur-35 in blood or tissue by liquid scintillation counting. Anal Bioch 16: 500–509, 1966.
15. Mahvi D, Bank H, Harley R. Morphology of a naphthalene-induced bronchiolar lesion. Am J Pathol 86: 559–571, 1977.
16. Malament DS, Shklar G. Inhibition of DMBA carcinogenesis of hamster buccal pouch by phenathrene and dimethylnaphthalene. Carcinogenesis 2: 723–729, 1981.
17. Melancon MJ, Lech JJ. Uptake, biotransformation disposition and elimination of 2-methylnaphthalene and naphthalene in several fish species. ASTM spec. Tech. Publ. STP 667, Aquat Toxicol 5–22, 1977.
18. Murata Y, Denda A, Maruyama H, Konishi Y. Chronic toxicity and carcinogenicity studies of 1-methylnaphtalene in B6C3F1 mice. Fund App Toxicol 21: 44–51, 1993.
19. Ott FS, Harris RP, O'Hara SCH. Acute and sublethal toxicity of naphthalene and three methylated derivatives to the estuarine copepod, *Eurytemora affinis*. Mar Environ Res 1: 49–58, 1978.
20. Plopper CH, Macklin J, Nishio S, Hyde D, Buckpitt A. Relationship of cytochrome P-450 activity to Clara cell cytotoxicity. Lab Invest 5: 553–565, 1992.
21. Rassmusen RE, Diem Hoąng Do, Tae Sun Kim. Comparative cytotoxicity of naphthalene and its monomethyl- and mononitro-derivatives in the mouse lung. J Appl Toxicol 6 (1): 13–20, 1986.
22. Reid W, Ilett K, Glick J, Krishna G. Metabolism and binding of aromatic hydrocarbons in the lung. Relationship to experimental bronchiolar necrosis. Am Rev Respir Dis 107: 539–551, 1973.

23. Saethre LJ, Falk-Petersen IB, Sydnes LK, Lonning S, Naley AM. Toxicity and chemical reactivity of naphthalene and methylnaphthalenes. Aquat Toxicol 5: 291–306, 1984.
24. Safe SH, Plugge H, Cittim B, Crocker JFS. Analysis of adjuncts used in formulations for the application of organophosphorus pesticides. Natl. Res. Council Can. Assoc. Comm. Sci. Criteria Environ Dual Publ (16073): 51–76, 1977.
25. Sandmeyer EE. Aromatic hydrocarbons. In: Patty's Industrial Hygiene and Toxicology. 3rd edition. Vol. 23. Clayton GD, Clayton FE, eds. John Wiley and Sons, New York 1981.
26. Schmeltz I, Tosk J, Hilfrich J, Hirota N, Hoffman D, Wynder EL. Bioassays of naphthalene and alkyl naphthalenes for co-carcinogenic activity. Relation to tobacco carcinogenesis. In: Carcinogenesis. Polynuclear Aromatic Hydrocarbons. Vol. 3. PW Jones, RI Freudenthal, eds. Raven Press, New York 1978.
27. Tong S, Hirokata Y, Trush M, Mimnaugh E. Lowe M, Gram T. Clara cell damage and inhibition of pulmonary mixed function oxidase activity by naphthalene. Biochem Biosphys Res Commun 100: 944–950, 1981.
28. Warren D, Brown D, Buckpitt A. Evidence for cytochrome P-450 mediated metabolism in the bronchiolar damage by naphthalene. Chem Biol Interact 40: 287–303, 1982.
29. Wesołowski W, Gromiec JP. Prediction of exposure to engine fuel vapours. Med Pr 47 (1): 19–29, 1996 (in Polish).

Received for publication: October 6, 1998
Approved for publication: October 30, 1998