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TISSUE DISTRIBUTION AND ELIMINATION OF SELECTED CHLORINATED NAPHTHALENES

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Abstract.
Objectives: Polychlorinated naphthalenes are widespread, persistent environmental pollutants. Commercial products are generally mixtures of several congeners and range from thin liquids to hard waxes of high melting point. The higher chlorinated naphthalene products are used as impregnants for condensers and capacitors and dipping encapsulating compounds in electronics. The aim of this study was to investigate the disposition of penta- and tetrachloronaphthalene in rats following a single intraperitoneal administration. **Materials and Methods:** Experiments were performed on male Outbred Wist rats with body weight of 200–250 g. Both compounds labeled with tritium, were given intraperitoneally in a single dose of 10 mg/kg body weight. Blood and selected tissues distribution of ^{3}H-radioactivity as well as urine and feces excretion from 0 to 336 h were traced following the administration. **Results:** After 120 h about 70% of the given dose was excreted in feces. Feces proved to be the main route of tritium excretion; only about 6% were excreted in urine within 120 h. In all the examined tissues, the highest ^{3}H concentrations were found in the fat tissue, liver, kidneys and adrenals. **Conclusions:** Following calculations of the balance of total tritium excreted and stored, it was found out that both chloronaphthalenes belong to compounds of slow turnover rate in the rat body, and especially in the case of repeated exposure they might accumulate in the body.

Key words:
Tetrachloronaphthalene, Pentachloronaphthalene, Distribution, Excretion, Rats

INTRODUCTION

Chlorinated naphthalenes are compounds based on the naphthalene ring system, where one or more hydrogen atoms have been replaced by chlorine. Commercial products are generally mixtures of several congeners and range from thin liquids to hard waxes of high melting point [1]. They are mainly used in cable insulation, wood preservation, engine oil additives, electroplating masking compounds, capacitors and refractive index testing oils and also as a feedstock for dye production [2].

Chlorinated naphthalenes, especially dioxin-like congeners, have been detected in adipose tissue, liver blood and breast milk samples of the general population at concentration within the ng/kg range. The dominating congeners in almost all human specimens examined were two penta- and two hexa-isomers, and to a lesser extent some tetra-isomers [3–7].

Some congeners of polychlorinated naphthalenes (PCNs) (four lateral chlorines at 2,3,6,7 i.e., congeners 2,3,6,7-tetrachloronaphthalene, 1,2,3,6,7-pentachloronaphthalene, 1,2,3,4,6,7-hexachloronaphthalene, 1,2,3,5,6,7-hexachloronaphthalene, 1,2,3,6,7,8-hexachloronaphthalene, 1,2,3,4,5,6,7-heptachloronaphthalene, and 1,2,3,4,5,6,7,8-octachloronaphthalene) similar to 2,3,7,8- tetrachloro-

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dibenzo-p-dioxin (TCDD), may exert toxicity comparable to the more toxic polychlorinated biphenyls (PCBs) [1].

Severe skin reactions and liver disease have both been reported after occupational exposure to PCNs. The clinical and toxicological symptoms of PCNs are very similar to those caused by PCBs, polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) [8].

Apart from chloracne, other systemic effects, in particular liver disease, have been reported after occupational exposure to PCNs [9,10]. All chlorinated naphthalenes tested caused skin irritation, and penta- and hexachlorinated naphthalenes showed hyperkeratotic activity in the rabbit ear test and in hairless mice, which is consistent with findings in cattle (bovine hyperkeratosis or X-disease) and humans (chloracne), e.g., liver disease have been reported after occupational exposure to chlorinated naphthalenes. Chloracne was common among workers handling chlorinated naphthalenes in the 1930s and 1940s [1]. Other symptoms described in workers exposed to PCNs included irritation of the eyes, fatigue, headache, anemia, hematuria, impotentia, anorexia, nausea, vomiting, occasionally severe abdominal pain. Systemic effects resulting in liver disease have been reported only due to inhalation of chloronaphthalenes [1].

Chlorinated naphthalenes, like related compounds, have been demonstrated to be the inducers of cytochrome P-450-dependent microsomal enzymes (CYP 1A1 – typical for dioxin-like compounds). Chlorinated naphtalenes were also found to change lipid peroxidation and antioxidant enzyme activities in rats in a manner indicative of increased oxidative stress [1].

Although experimental data are limited, the kinetic behavior of PCNs resembles that of related polyhalogenated aromatic compounds, which can be absorbed via oral, dermal, and inhalation routes. As with these classes of substances, the lower chlorinated PCNs are less persistent in the body than the more highly chlorinated ones. Accordingly, metabolism and the main route of elimination (via feces or urine) seem to be influenced by the degree of chlorination [1].

The aim of the present study was to investigate the kinetics of body distribution and excretion of penta- and tetrachloronaphthalene in rats following a single, intraperitoneal administration.

MATHERIALS AND METHOD

Chemicals

1,2,3,4–tetrachloronaphthalene–ring–U–^{3}H, with a specific activity of 707 MBq/g, unlabeled 1,2,3,4–tetrachloronaphthalene, 1,2,3,4,5–pentachloronaphthalene ring–U–^{3}H, with a specific activity of 474 MBq/g and unlabeled 1,2,3,4,5–pentachloronaphthalene each chromatographically pure, were obtained from the Institute of Radiation, Faculty of Chemistry, Technical University of Łódź, Poland. All the other chemicals (Merck, Germany; Loba Feinchemie, Germany) were of analytical grade.

Animals

Adult male Outbred Wist (Rattus) rats of 200–250 g body weight (b.w), were obtained from the breeding colony of the Nofer Institute of Occupational Medicine in Łódź. The animals were supplied at least 1 week before the experiment and were fed a standard palletized diet Murigram (Agropol, Matycz, Poland) and had free access to water.

Animal treatment

The animals were placed individually in glass metabolism cages (Simax, the former Czechoslovakia) and allowed to acclimatize for 2 days (48 h). Subsequently, rats (groups n = 6 or 8) were administered, intraperitoneally, a single dose of 10 mg/kg of [^{3}H]–1,2,3,4-tetrachloronaphthalene (about 0.8 MBq per animal) and [^{3}H]–1,2,3,4,5–pentachloronaphthalene (about 0.7 MBq per animal) both dissolved in olive oil. Immediately after administration, the rats were placed individually into glass metabolism cages, which enabled the collection of separate samples of urine and feces.

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

Blood samples were collected from the tail veins of eight rats using calibrated, heparinized capillaries (0–336 h) following a single administration of the compounds; 0.03 ml of blood was collected each time. Rats were decapitated

under light-ether narcosis at appropriate time intervals and examined tissues were removed for the determination of radioactivity. In all the experiments, the Polish law on the protection of animals was followed [11]. The kinetics of tritium activity in blood was carried out using SIGMA PLOT 3.0 (Jandel Corporation) for WINDOWS. Tissue homogenates (20%), feces water homogenates (10%) and erythrocytes were digested according to the method of Mahin and Lofberg (1966) [12]. Urine samples, diluted with water to 50 ml, and plasma samples were measured directly. All radioactivity measurements were carried out using Racbeta 1209 (LKB, Sweden), liquid scintillation counter and Hydroluma from Baker (Germany) as the scintillation mixture. Counting correction was achieved using the external standard method.

RESULTS

The excretion of tritium after a single intraperitoneal administration of 1,2,3,4-tetrachloronaphthalene and 1,2,3,4,5-pentachloronaphthalene at the doses of 10 mg/kg is presented in Tables 1 and 2.

As indicated, feces proved to be the main route of ^{3}H excretion. The excretion was similar for the investigated compounds. However, pentachloronaphthalene was excreted faster (over 40% of the administered dose after 24 h), whereas comparable amount of tetrachloronaphthalene was excreted after 48 h. Altogether, after 5 days nearly 65% of tetrachloronaphthalene dose and 70% of pentachloronaphthalene dose were excreted in feces.

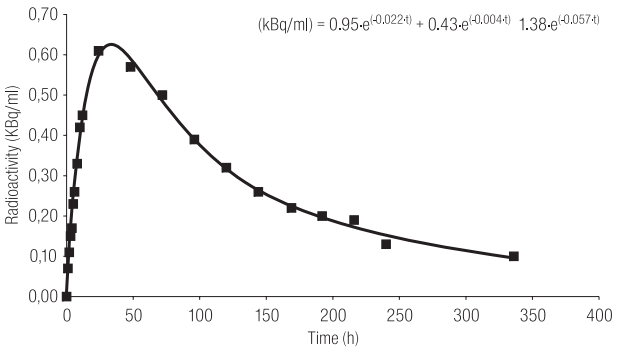

Fig. 1. The kinetics of ^{3}H distribution in plasma following a single i. p. administration of 1,2,3,4-tetrachloronaphthalene at a dose of 10 mg/kg (800 kBq/rat) in rats (Results are expressed as the mean from 8 rats, SD ≤ 20%).

Table 1. Total balance of tritium following a single, i.p. administration of 1,2,3,4-tetrachloronaphthalene-[ring-U-^{3}H] at a dose of 10 mg/kg (800 kBq/rat) The values are expressed as the percent of administered dose. Explanation: all results are expressed as the mean from five rats ± SD; to assess ^{3}H distribution, blood was accepted as 7 ml per 100 g b.w., fat tissue, 12% and muscles, 40% of the whole body weight [13]

Medium	Time following the administration Dose (%)		
	0–24 h	0–48 h	0–120 h
Urine	2.65 ± 0.009	4.26 ± 0.008	5.68 ± 0.02
Feces	15.97 ± 2.56	41.97 ± 2.46	64.34 ± 1.71
Blood	1.91 ± 0.12	1.76 ± 0.10	0.80 ± 0.04
Fat tissue	44.81 ± 4.54	36.62 ± 8.02	2.51 ± 0.36
Muscles	5.63 ± 1.13	6.06 ± 0.59	4.24 ± 0.97
Liver	1.91 ± 0,58	1.09 ± 0.09	0.53 ± 0.07
Remaining tissues	0.81 ± 0.05	0.35 ± 0.02	0.21 ± 0.01
Total	73.69	92.11	78.31

Table 2. Total balance of tritium following a single i.p. administration of 1,2,3,4,5-pentachloronaphthalene-[ring-U-^{3}H] at a dose of 10 mg/kg (700 kBq/rat) The values presented are expressed as the percent of administered dose. Explanation: all results are expressed as the mean from six rats ± SD; to assess ^{3}H distribution, blood was accepted as 7 ml per 100 g b.w., fat tissue, 12% and muscles, 40% of the whole body weight [13]

Medium	Time following the administration Dose (%)		
	0–24 h	0–48 h	0–120 h
Urine	1.90 ± 0.98	3.57 ± 0.81	6.71 ± 0.62
Feces	41.40 ± 17.65	59.42 ± 11.80	71.27 ± 5.16
Blood	2.11 ± 0.23	1.99 ± 0.15	1.08 ± 0.06
Fat tissue	40.01 ± 8.26	19.16 ± 2.16	3.00 ± 1.42
Muscles	11.06 ± 0.90	12.41 ± 0.60	6.89 ± 1.80
Liver	2.26 ± 0.20	2.22 ± 0.15	1.70 ± 0.35
Remaining tissues	0.86 ± 0.07	0.78 ± 0.11	0.41 ± 0.07
Total	99.60	99.55	91.11

The kinetics of accumulation and decline of ^{3}H radioactivity in blood plasma during 0–336 h following tetra- and pentachloronaphthalene administration at a dose of 10 mg/kg are presented in Figs. 1 and 2. The maximum level of plasma tritium was observed 12 h after administration of tetrachloronaphthalene and 24 h after administration of pentachloronaphthalene.

The accretion of ^{3}H in plasma proceeded with kinetic constant of 0.078 h^{-1} for tetrachloronaphthalene and 0.057 h^{-1} for pentachloronaphthalene. Decline of ^{3}H for tetrachlo-

ronaphthalene and pentachloronaphthalene in plasma were biphasic (Figs. 1 and 2) and half-lives for phase I for tetrachloronaphthalene were about 13 h and for pentachloronaphthalene about 32 h. Half-lives for phase II for both compounds were about 173 h.

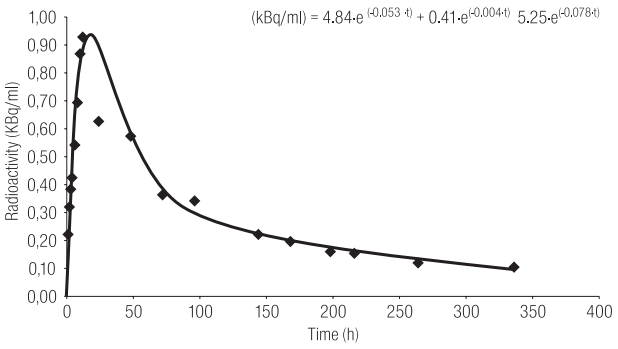

Fig. 2. The kinetics of ^{3}H distribution in plasma following a single i.p. administration of 1,2,3,4,5-pentachloronaphthalene at a dose of 10 mg/kg (700 kBq/rat) in rats (Results are expressed as the mean from 8 rats, SD ≤ 20%).

The tissue and organ distribution of tritium after a single i.p. administration of tetra- and pentachloronaphthalene is shown in Figs. 3 and 4. In all the examined tissues the highest levels of tritium accumulation were found after 12 h for tetrachloronaphthalene and after 24 h for pentachloronaphthalene.

The highest levels of tritium for both tetra- and pentachloronaphthalene were found in the fat tissue (a piece of abdominal fat), liver, kidneys and adrenals. The attempted calculations of the total balance of ^{3}H retained in the rat for both compounds are presented in Tables 1 and 2.

Quantitative calculations of tritium deposited in tissues should be treated as approximate values due to the fact

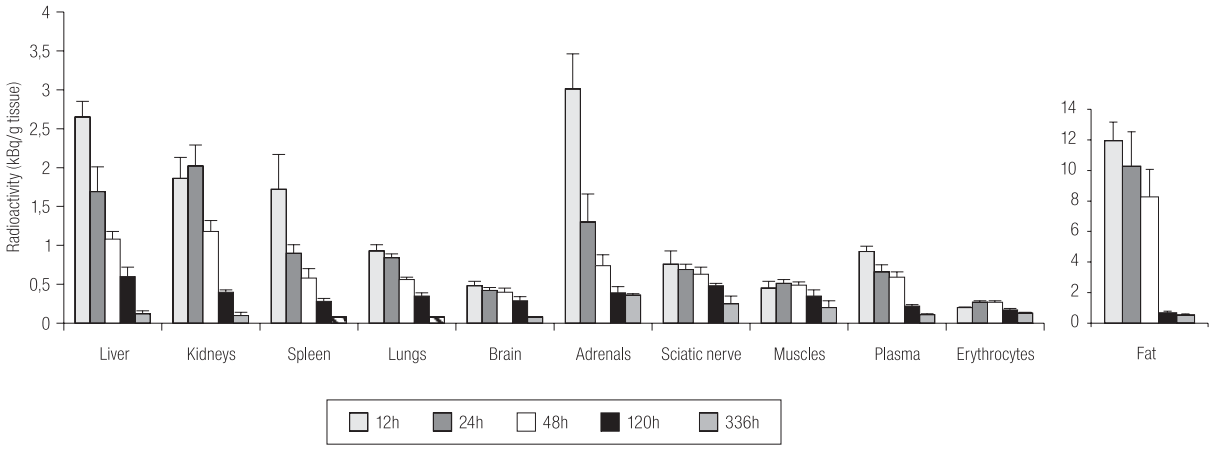

Fig. 3. The specific activity of ^{3}H in the tissue after i.p. administration of 1,2,3,4-tetrachloronaphthalene-[ring-U-^{3}H] at a dose of 10 mg/kg (800 kBq/rat).

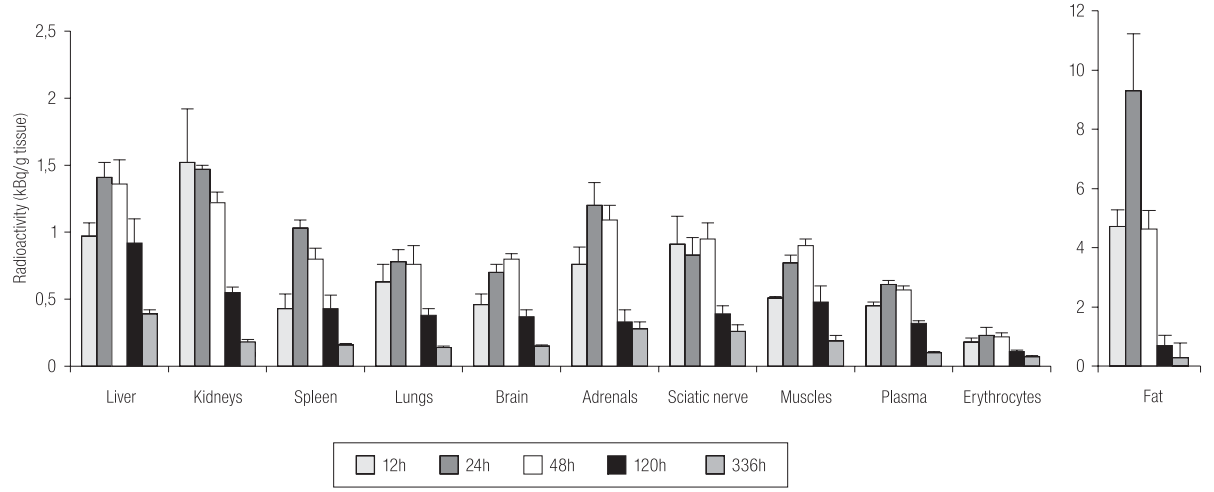

Fig. 4. The specific activity of ^{3}H in the tissue after i.p. administration of 1,2,3,4,5–pentachloronaphthalene-[ring-U-^{3}H] at a dose of 10 mg/kg (700 kBq/rat).

that they are burdened with some error, resulting from theoretical assumptions on the percentage share of muscular and fatty tissues with relation to the total body mass of rat. Tritium radioactivity was measured in selected tissues and organs, and thus it was not measured in the alimentary tract and its contents, skin, bones or tendons.

After 24 h, when the excretion in the feces was not yet completed, the total balance of ^{3}H retained in the body and excreted feces was about 74% of the given dose for tetrachloronaphthalene and almost 100% for pentachloronaphthalene.

DISCUSSION

Polychlorinated naphthalenes have been detected in the human adipose tissue, liver, blood and breast milk at concentrations in the range of μg/kg lipid range [3,5]. In the liver and adipose tissue samples of the general population maximum concentrations for total PCNs (tetra- to hexa-) have been found as high as 26.113 and 17.0 μg/kg, respectively [1,14].

Chlorinated naphthalenes can be absorbed via oral, inhalative and dermal routes, with absorption and distribution over the whole body after administration. The main target organs are liver and fat tissue both showing a high retention, especially for higher chlorinated congeners such as penta- or hexachloronaphthalenes. Half-lives of hexachloronaphthalene were calculated to be about 41 days in adipose tissue and 26 days in the liver of rats [1]. Although experimental data are limited, the kinetic behavior of PCNs resembles that of related to polyhalogenated aromatic compounds (e.g., PCDFs/PCDDs, PCBs), which can be absorbed via oral, dermal and inhalation routes. As with these classes of substances, the lower chlorinated PCNs are less persistent in the body than more highly chlorinated ones. Accordingly, metabolism and the main route of elimination (via feces or urine) seem to be influenced by the degree of chlorination [15–19].

Chu et al. [15–17] demonstrated that the tissue distribution obtained in his study may be caused by the substituted position of chlorine, administration route and species [18]. The different distribution between dichloronaphthalenes and octachloronaphthalenes may be attributed to the number and substitution of chlorine [19]. Higher chlorinated naphthalenes were metabolized more slowly than lower chlorinated PCN as PCB was indicating that higher chlorinated naphthalenes have longer biological half-lives than lower chlorinated naphthalenes [19]. It is suggested that a prolonged accumulation of octachloronaphthalene in the body may be a reason for the higher toxicity in comparison to naphthalenes with lower number of chlorine atoms.

This report presents data on the tissue distribution and excretion of 1,2,3,4-tetrachloronaphthalene and 1,2,3,4,5-pentachloronaphthalene obtained by radiotracer studies in rats. Our results of body distribution of tetra- and pentachloronaphthalene in rats are similar to the results obtained by Jacobsson et al. [20]. Only insignificant differences were observed in the rate of the total excretion of both compounds in feces after 24 h. In the case of tetrachloronaphthalene the excretion was lower, about 16% of the given dose, whereas for pentachloronaphthalene it was higher, nearly 42%. Altogether, after 5 days about 70% of the dose of both administered compounds was excreted. Since the compounds were administered intraperitoneally, it seems likely that all the amount being found in feces were excreted in bile.

Elimination and distribution of ^{14}C-PCN mixture containing three tetrachloronaphthalene (45%), six pentachloronaphthalene (30%), and four hexachloronaphthalene (10%) isomers have been studied in female Sprague-Dawley rats by Jacobson et al. [20]. Five days after oral dosing with the ^{14}C-PCN mixture (0.58 mmol/kg b.w. in peanut oil), about 94% of the totally recovered radioactivity (from absorbed and unabsorbed material) was found in feces. The total urinary excretion was 4.3%. Within tissues, the highest concentrations of radioactivity were observed in liver and abdominal fat (about 10 pmol/mg fresh weight each), followed by the kidney (about 3 pmol/mg fresh weight) and lungs, blood plasma, and adipose tissue (about 1.5 pmol/mg fresh weight each) [20].

It results from the studies on tissue distribution that both investigated compounds, due to their lipophilic nature, have high affinity mainly to fatty tissue in which after 48 h tetrachloronaphthalene presented over 35% of the ad-

ministered dose, whereas pentachloronaphthalene nearly 20%. It is known from the studies on biotransformation of similar group of chlorinated compounds (PCBs) that lower chlorinated isomers were rapidly metabolized and excreted in comparison with the other isomers, which were slowly metabolized and excreted with much longer half-lives. The results indicated that the metabolism of higher chlorinated compounds is slow, when the position of the chlorine atoms is such that arene oxide formation is inhibited. The metabolic data on individual isomers show that at least up to hexachloro-compounds, ordinary hydroxylation can occur. It is reasonable to consider that it is not only a consequence of poor metabolism pronounced by persistence of pentachloro- and hexachloro-compounds in the tissue, but rather of their delayed metabolism, because they are sequestered from tissues, in which the bulk of metabolism takes place. This is supported by Matthews and Anderson [21] who showed that in animals caused to lose a substantial portion of body weight, a higher storage of chlorinated compounds can indeed be metabolized.

In conclusion, both tested compounds (tetra- and pentachloronaphthalene) have a slow turnover rate in the rat and, especially in the case of repeated exposure, might accumulate in the body and form long-term storage compartments.

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