THE EFFECT OF EXPOSURE ROUTE ON THE DISTRIBUTION AND EXCRETION OF HEXACHLORONAPHTHALENE IN RATS

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Abstract

Objectives: Polychlorinated naphthalenes (PCNs), like other persistent organic pollutants (POPs), are widespread, global environmental contaminants. These compounds still represent a great environmental problem, mostly because of the risk of secondary air pollution. They are characterized by long durability and tendency to bioaccumulate, which means that they are practically ubiquitous in all environmental media and ecosystems. The aim of this study was to investigate the distribution and excretion of hexachloronaphthalene (HxCN) in rats following a single intraperitoneal or intragastrical administration. **Materials and Methods:** Experiments were performed on male outbred Wistar rats with body weight of 220–240 g. They were given [^{14}C]-HxCN intraperitoneally (i.p.) or intragastrically (p.o.) in a single dose of 0.3 mg (150 kBq) per rat. The distribution of radioactivity in blood and selected organs or tissues, as well as urine and faeces excretion were traced following the administration. **Results:** The decline of [^{14}C]-HxCN in plasma was biphasic and the calculated half-lives for phases I and II were ~6 and 350 h, respectively. Following 120 h after administration, ~51% (intragastrical) and ~34% (intraperitoneal) of the dose were excreted with faeces. Regardless of the administration route, the highest HxCN concentrations were found in liver and adipose tissue, where the compound showed high retention: the highest retention in liver was found 24 h after intragastrical (32%) and intraperitoneal (38%) administration while in adipose tissue ~30% retention was observed 120 h after HxCN administration regardless of its route. **Conclusions:** Following the calculation of the balance of total [^{14}C]-HxCN excreted and stored, it was found that hexachloronaphthalene belongs to the compounds of a slow turnover rate, and in the case of repeated exposure it may accumulate in the rat body.

Key words:
Hexachloronaphthalene, Distribution, Excretion, Rat

INTRODUCTION

Polychlorinated naphthalenes (PCNs) comprise a group of compounds, consisting of naphthalene substituted with 1–8 chlorine atoms, yielding 75 possible congeners [1]. They were previously used for a variety of industrial purposes owing to their dielectric, water-repellent, flame-retardant, and anti-fungal properties [1]. PCNs have also been found in polychlorinated biphenyl (PCB) formulations [2] and combustion-related emissions, such as those of waste incinerators [3,4]. Mixtures of PCNs have been produced in several countries under the trade names Halowax, Nibren and Seekay waxes [1,3,5]. Production figures for PCNs are not well known, but have been estimated to be about 10% of the global PCB production [5]. Despite the fact that the manufacture of PCNs was finally stopped in the 1980s, they are still present in

the environment due to their persistent, lipophilic properties [6]. It is estimated that 1.0 tons of PCNs were emitted in Europe in 2000, as a result of waste and other combustions, industrial sources, solvent and product usage [5].

Polychlorinated naphthalenes, like other persistent organic pollutants (POPs), are widespread global environmental contaminants and they still represent a great environmental problem, mostly because of the risk of secondary air pollution [4,5,7–9]. They are characterized by long durability and tendency to bioaccumulate, which means that they are practically ubiquitous in all environmental media and ecosystems [3,10–14]. These pollutants have been detected in air, water, sediments, soils and biota [15–17].

Food contamination is one of the most serious consequences of the natural environmental contamination by PCNs. Consumption of contaminated food is the major source of human exposure to PCNs [18]. The studies carried out by different researchers show that PCNs, especially hexachloronaphthalene (HxCN), have been found in food of animal origin (primarily in fish, but also in milk, meat and eggs) [6,12,14,17,19–21]. Foods that were subject to some measure of processing (oils, cereals, meat etc.) and fish showed highest levels of PCNs [22,23].

Thus, it is not surprising that PCNs have been found in humans and wildlife. General population studies carried out in different regions of the globe have revealed the highest concentrations of PCNs (mostly penta- and hexachloronaphthalene isomers) in liver and adipose tissue [1,4,24,25]. The Swedish studies performed in 1972–1992 revealed the presence of these compounds (including mainly 1,2,3,5,6,7-HxCN) in breast milk, which may be particularly dangerous to the developing infants [26], mostly because the elimination of compounds like PCNs and their lipophilic metabolites from body fat is very slow [1].

The literature review shows that data on the disposition of single PCN congeners in the rat body, including a comprehensive assessment of their absorption, distribution and excretion are rather scarce. The majority of the reported experiments have been performed for the commercially available mixtures of PCNs [1]. Considering that individual congeners of the discussed compounds may significantly differ in their distribution and affinity to the critical organs in general and liver in particular, the toxicokinetics of hexachloronaphthalene, one of the most toxic [27–29] and the most intensely bioaccumulative [14,30] congeners, was investigated in this study.

The aim of this study was to investigate the distribution and excretion of hexachloronaphthalene in rats following a single intraperitoneal or intragastrical administration.

MATERIALS AND METHODS

Chemicals
^{14}C-labelled hexachloronaphthalene [^{14}C]-HxCN with a specific activity of 500 MBq/g was obtained from the Institute of Radiation, Faculty of Chemistry, Technical University of Łódź, Poland as a mixture of isomers [^{14}C]-HxCN (over 94%) and it contained mainly [^{14}C]-1,2,3,5,6,7-HxCN (81%). [^{14}C]-HxCN was synthesized by dechlorination of [^{14}C]-octaCN [31]. Other chemicals used were of the highest grade commercially available.

Animals
Adult male Wistar rats (aged eight weeks) weighing 220–240 g from the breeding colony of the Medical University, Łódź, were used in the experiments.

The animals were supplied at least one week before the experiment and were fed a standard pelletized diet (Murigram from Agropol, Motycz, Poland) and had free access to water. The exposed rats were housed five per cage (cage size, 35×55×25 cm) at a room temperature of 21–23°C, and relative humidity of 55±5%. The animals were maintained in a constant light/dark cycle of 12/12 h.

Animal treatment

[14C]-HxCN was dissolved in sunflower oil and administered to the male rats (5 animals/group were used per one time point) intraperitoneally (i.p.) or intragastrically (p.o.) via a metal gastric tube in a single dose of 1.3 mg/kg body weight (b.w.) and about 0.3 mg (150 kBq) per animal in a volume of 0.5 ml/rat. After the exposure to [14C]-HxCN, the animals were housed in individual metabolic cages (Simax, the former Czechoslovakia) and kept for five days (120 h) to enable separate collection of urine and faeces. In accordance with OECD guidelines, body mass of the animals, as well as fodder and water consumption were controlled every day.

Sampling of biological material and measurements of radioactivity

Blood samples were collected from the tail veins of eight rats using calibrated, heparinized capillaries following only a single intraperitoneal administration of the compound; 0.03 ml of blood was collected each time (1–432 h). The kinetics of [14C]-HxCN activity in plasma was determined using the Jandel Co. SIGMA PLOT 3.0 for WINDOWS software.

Rats were decapitated at appropriate time intervals and the tissues were removed for the determination of radioactivity. Tissue water homogenates (20%), faeces water homogenates (10%) and blood were digested according to the method of Mahin and Lofberg [32].

Body composition estimates for blood and muscle were 7% and 40% [33,34], and 12% for adipose tissue [35]. Urine samples, diluted with water to 25 ml, and plasma samples were measured directly. All radioactivity measurements were carried out using a Racbeta 1209 (LKB, Sweden), liquid scintillation counter and EcoLume™ from MP Biomedicals, LLC (USA) as the scintillation mixture. Counting correction was achieved using the external standard method.

ETHICS

All experimental procedures were carried out according to the Directive of the European Parliament and of the Council (2010/63/UE) on the protection of animals used for scientific purposes. The investigations were performed with the permission of the Local Ethical Committee for Experimentation on Animals of Łódź, Poland (No 42/ŁB 478/2009).

RESULTS

Intraperitoneal administration

The decline in plasma

The kinetics of radioactivity declined in blood plasma within 1–432 h after a single i.p. administration of [14C]-HxCN; it was expressed by the following equation: $A = 0.24e^{-0.119t} + 0.08e^{-0.002t}$; which means that the decline of [14C]-HxCN in plasma was biphasic and the calculated half-lives for phases I and II were about 6 and 350 h, respectively.

The excretion

The results of [14C]-HxCN excretion within 24–120 h, following a single i.p. administration at a dose of 0.3 mg/rat (150 kBq/animal) are summarized in Table 1.

After i.p. administration of the compound, its excretion via faeces (the main excretion route) was slow; in total, 34% of the received HxCN dose were excreted after five days. A 24-h excretion of HxCN in faeces during five days of sampling was comparable, about 6% of the received dose/day (6.57±1.62). Urinary excretion of HxCN was marginal, its total value after five days was about 1% of the received dose.

Tissue distribution

The tissue and distribution of [14C]-HxCN radioactivity in the individual organs and tissues, expressed in terms

Table 1. Excretion of [^{14}C]-HxCN-derived radioactivity in urine and faeces, expressed as percentage of total dose after a single intraperitoneal administration of 0.3 mg [^{14}C]-HxCN per rat (150 kBq/rat)*

| Medium	Time following the administration (h)				
	0–24	24–48	48–72	72–120	0–120
Urine	0.33±0.04	0.22±0.05	0.10±0.05	0.30±0.12	0.95±0.07
Faeces	5.03±0.93	8.25±0.74	6.42±0.79	14.09±0.98	33.79±1.81
Total	5.36±0.89	8.47±0.65	6.52±0.82	14.39±1.05	34.74±0.94

* The values are presented as mean ±SD for five rats.

of kBq/g tissue within 24–504 h after the single i.p. administration is shown in Figure 1.

Referring now to Figure 1, the highest [^{14}C]-HxCN concentrations were found in the liver and in the adipose tissue. Liver HxCN concentration was by several times higher than that in the abdominal adipose tissue. Liver/Adipose (L/A) concentration ratio values were: 3.6 after 24 h; 3.0 after 48 h; 2.5 after 72 h; 1.7 after 120 h; and 1.4 after 504 h. In the remaining tissues and organs, [^{14}C]-HxCN concentration values measured within 24–504 h after administration were at least 10 times lower compared with the corresponding values for the liver and adipose tissues. The maximum value of [^{14}C]-HxCN radioactivity in liver, spleen, blood and muscles was observed 24 h, and in other organs 120 h after HxCN administration. At the final time point (504 h after administration) [^{14}C]-HxCN concentrations were significantly decreased in the majority of tissues and organs tested, except for brain, sciatic nerve and blood. In brain and sciatic nerve no downward trend in HxCN levels were observed even 504 h after the administration.

Total balance of [^{14}C]-HxCN deposited in the tested organs (tissues) and excreted from the body within 24–120 h following a single i.p. administration is presented in Table 2. Liver and adipose tissue played a major role in the balance of [^{14}C]-HxCN deposited in the body (about 38% and 35% of the received dose, respectively) from the first day after the compound administration. Within first three days, about 75% of the administrated dose were accumulated in all tested organs and tissues. Five days (120 h) after HxCN administration, the body retention was still very high, over 58%; however, the compound deposits in the liver were already significantly lower (by over 50% compared with those after 24 h). No similar effect could be observed in adipose tissue, where the level of the deposited compound was maintained at the
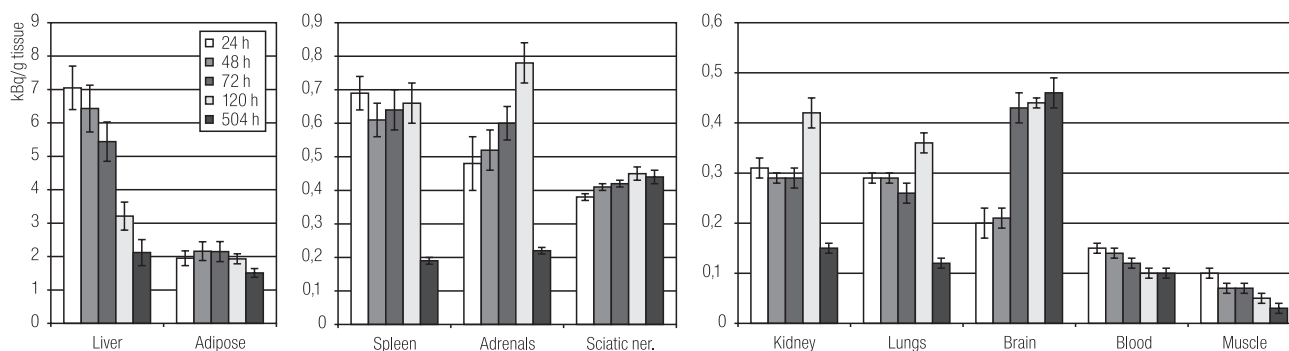
Each point represents the mean ±SD of five animals.

Fig. 1. Specific radioactivity of [^{14}C]-HxCN in the tissue after a single intraperitoneal administration [^{14}C]-HxCN of 0.3 mg (150 kBq)/rat

Table 2. Total balance of [^{14}C]-HxCN-derived radioactivity expressed as percentage of total dose after a single intraperitoneal administration of 0.3 mg [^{14}C]-HxCN per rat (150 kBq/rat)*

Medium	Time following the administration (h)				
	24	48	72	120	504
Liver	37.58±4.04	34.27±3.05	29.07±3.49	17.74±4.49	13.30±2.47
Adipose tissue (abdominal)	34.37±5.68	39.75±5.08	39.25±5.43	34.68±3.94	28.99±2.50
Muscles	5.09±1.04	4.21±1.12	4.30±0.95	3.91±0.88	1.92±0.64
Blood	1.64±0.28	1.49±0.18	1.32±0.10	1.30±0.10	1.12±0.11
Remaining tissues	0.59	0.52	0.54	0.47	0.28
Total in tissues	79.27	80.24	74.48	58.10	45.74
Excretion (h)	0–24	0–48	0–72	0–120	not determined
urine	0.33±0.04	0.55±0.05	0.65±0.06	0.95±0.07	
faeces	5.03±0.93	13.28±1.23	19.70±1.30	33.79±1.81	
Total balance	84.63	93.55	94.29	92.37	

* The values are presented as mean ±SD for five rats.

same level (over 35%). In total, 21 days (504 h) after administration, body retention of [^{14}C]-HxCN was about 46%, of which ~13% were found in liver and ~29% in adipose tissue. The total balance of HxCN deposited in organs and excreted from the body after five days was about 93% of the administered dose, of which about 35% were excreted.

Oral administration

The excretion

The results of [^{14}C]-HxCN excretion within 24–120 h following a single p.o. administration at a dose of 0.3 mg/rat (150 kBq/animal) are summarized in Table 3.

After p.o. administration, faeces were the main route of HxCN elimination and, following five days, over 51% of the administered dose were excreted. The first 24 h were the most important for the balance of HxCN elimination (about 34% of the total dose).

Like after i.p. administration, HxCN excretion in urine was of marginal significance; less than 1% of the total dose was excreted via this route after five days.

Tissue distribution

Figure 2 shows the distribution of [^{14}C]-HxCN radioactivity in selected tissues and organs, in terms of kBq/g tissue,

Table 3. Excretion of [^{14}C]-HxCN-derived radioactivity in urine and faeces, expressed as percentage of total dose after a single intragastrical administration of 0.3 mg [^{14}C]-HxCN per rat (150 kBq/rat)*

Medium	Time following the administration (h)				
	0–24	24–48	48–72	72–120	0–120
Urine	0.18±0.03	0.12±0.02	0.10±0.05	0.22±0.09	0.62±0.06
Faeces	34.02±1.53	9.25±2.34	3.45±0.89	4.49±1.05	51.21±4.51
Total	34.20±0.39	9.37±2.48	3.55±1.02	4.71±0.55	51.83±4.24

* The values are presented as means ±SD for five rats.
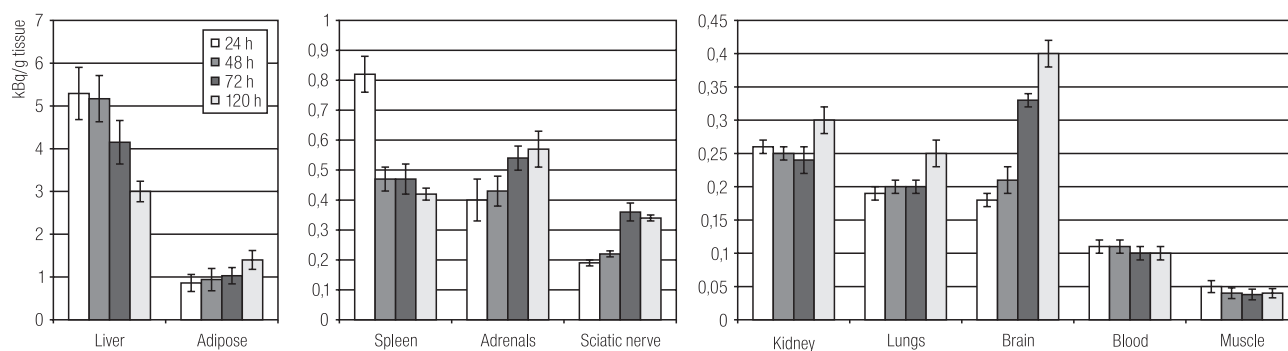
Each point represents the mean ±SD of five animals.

Fig. 2. Specific radioactivity of [14C]-HxCN in the tissue after a single intragastrical administration [14C]-HxCN of 0.3 mg (150 kBq)/rat

within 24–120 h after a single p.o. administration. Like after i.p. administration, the highest [14C]-HxCN concentrations were observed in liver and in adipose tissue. HxCN concentration was several times higher in the liver than in the abdominal adipose tissue and the calculated L/A ratio was 6.22 after 24 h, 5.5 after 48 h, 3.9 after 72 h and 2.1 after 120 h.

Maximum [14C]-HxCN radioactivity in liver and spleen was found already 24 h after its administration. In other tissues and organs, the highest concentrations of the compound were observed 120 h after administration. In liver and spleen, unlike in other organs, HxCN concentrations significantly decreased (about 50%) after 120 h as compared with the maximum level after 24 h.

Total balance of [14C]-HxCN deposited in the tested organs (tissues) and eliminated from the body within 24–120 h, following a single p.o. administration, is shown in Table 4.

Table 4. Total balance of [14C]-HxCN-derived radioactivity expressed as percentage of total dose after a single intragastrical administration of 0.3 mg [14C]-HxCN per rat (150 kBq/rat)*

Medium	Time following the administration (h)			
	24	48	72	120
Liver	31.74±5.18	27.60±4.35	23.57±3.39	16.04±3.00
Adipose tissue (abdominal)	16.17±4.08	16.98±5.58	19.20±3.83	26.10±4.54
Muscles	3.15±0.84	2.46±0.90	2.31±0.65	2.60±0.58
Blood	1.34±0.09	1.20±0.10	0.90±0.07	0.95±0.08
Remaining tissues	0.40	0.37	0.38	0.36
Total in tissues	52.80	48.61	46.36	46.05
Excretion (h)	0–24	0–48	0–72	0–120
urine	0.18±0.03	0.30±0.03	0.40±0.05	0.62±0.06
faeces	34.02±1.53	43.25±3.83	46.72±4.24	51.21±4.51
Total balance	87.00	91.16	93.48	97.88

* The values are presented as mean ±SD for five rats.

Like in i.p. administration, liver and adipose tissue proved to be the most essential for the balance of [^{14}C]-HxCN deposited in the body. Almost 32% of total dose was accumulated in the liver after 24 h. Five days after administration, the level of HxCN deposited in this organ was 16%, a value similar to that observed after i.p. administration. After the same period of time [^{14}C]-HxCN body retention was over 46% of the total dose, of which about 26% of the compound was deposited in adipose tissue. Total balance of [^{14}C]-HxCN retained and excreted from the rat body 120 h after administration was almost 98 %, of which about 52 % were excreted.

Quantitative calculations of [^{14}C]-HxCN deposited in tissues after both i.p. and p.o. administrations should be regarded as approximate values due to the fact that they may be encumbered with an error resulting from the adopted theoretical assumptions about the proportion of the muscular and fatty tissues relative to the total body mass of the rat. [^{14}C]-HxCN 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.

DISCUSSION

Polychlorinated naphthalenes have been detected in human adipose tissue samples [1,24,36,37], liver [24], blood [38,39] and breast milk [26,40,41] of the general population.

The PCN congener/isomer pattern found in human samples was significantly different from that in the commercial PCN mixtures [1]. The dominating congeners in almost all human specimens examined were two penta- and two hexa- isomers, namely 1,2,3,5,7/1,2,4,6,7-pentachloronaphthalene and 1,2,3,4,6,7/1,2,3,5,6,7-hexachloronaphthalene [24,26,30,36,37,39].

Toxicological symptoms induced by PCNs are very similar to those caused by polychlorodibenzodioxins (PCDDs) [27,28]. The studies performed on rats by

Kilanowicz et al. [29,42,43] apparently confirm this observation. They show that PCNs, like dioxins, are very strong inducers of CYP1 A in liver [29,42,44]. They also induce anorectic effect leading to cachexy [29,42,44], promote teratogenicity (cleft palate and hydronephrosis) and are fetotoxic in the absence of maternal toxicity [43]. The similarity in the toxic effect between PCNs and TCDD has also been reported by Omura et al. [45]. They have provided evidence that gestational administration of 1,2,3,4,6,7-hexachloronaphthalene already in a very low dose (1 μg/kg b.w.) to pregnant rats has been found to accelerate the onset of spermatogenesis in male offspring.

Bearing in mind that toxic effects the compound exerts on the body depend not only on its concentration (dose) but also on the time of its action, the analysis of toxicokinetics may prove to be essential for the elucidation of the mechanism by which the toxic effect is exerted. It is also important to find out which PCN congener is most bioaccumulative and which organ can be regarded as critical.

Although experimental data are limited, the toxicokinetics of PCNs resembles that of related polyhalogenated aromatic compounds e.g., TCDD, which can be absorbed via oral, dermal, and inhalation routes. As with these classes of substances, the lower chlorinated naphthalenes are less persistent in the body than the more highly chlorinated ones.

The literature review shows that the majority of comparative studies on kinetics, including elimination and distribution, have been performed for PCN technical mixtures [30,46,47]. But there are only a few studies concerning tissue distribution and elimination of selected polychlorinated naphthalenes, e.g. tetra- and pentachloronaphthalene [48]. Only one examination was performed for hexachloronaphthalene retention in liver and adipose tissue; however, its distribution in other organs or its excretion was not investigated [30].

In view of the fact that hexachloronaphthalene is regarded not only as the most bioaccumulative [30] but also as probably

the most toxic amongst all PCNs [1], this work presents data on the tissue distribution and excretion of [^{14}C]-HxCN in rats. To compare and comprehensively assess absorption, distribution and excretion, the rats were administered [^{14}C]-HxCN via two routes: intraperitoneal and intragastric.

Our experiment provides firm evidence that hexachloronaphthalene, regardless of the administration route, intensively accumulates not only in adipose tissue, but also in liver, which makes it different from other PCN congeners, such as e.g. tetra- or pentachloronaphthalene [48]. The data calculated from kinetic equation indicating HxCN decline in plasma after its single intraperitoneal administration points to a possible accumulation of HxCN and presents the distribution of the compound in the rat body using a simplified mathematical model.

Adopting a two-compartment open model, the equation-based calculation of half-lives for HxCN were about 6 h for phase I, known as the distribution phase that indicates rapid and effective absorption from the peritoneal cavity, and 350 h for phase II, known as the excretion phase that suggests a possible body accumulation after repeated exposure.

To assess the efficacy of HxCN absorption from the alimentary tract on the basis of its excretion in faeces (main excretion route) and retention in the body, the administration routes, p.o. and i.p., were compared. The comparison showed that 120 h after p.o. administration, the total excretion of the compound in faeces accounted for about 52%, of which 34% was excreted after first 24 h; most likely this was the non-absorbed pool.

Owing to the fact that 24 h after p.o. administration 53% of the received dose were retained in the body and the total balance of HxCN, including the excreted quantity, was about 88%, it may be assumed that absorption from the alimentary tract was about 60–70%. This assumption seems to be confirmed by the outcome of the excretion balance after i.p. administration when total excretion with faeces was only 34% of the received dose after five days, while at the same time point HxCN retention in the body

was only insignificantly higher (by about 12%) than that after p.o. administration (about 58% of the dose).

Our experiment also demonstrated that, regardless of the route of [^{14}C]-HxCN administration, the highest concentrations, expressed as kBq/g tissue, were found first in liver and then in adipose tissue, while the concentration in liver was several-fold higher than that in adipose tissue. For example, the concentration ratio for liver/adipose tissue 24 h after p.o. administration of HxCN was 6.22 and was comparable with that determined by Asplund et al. [30]. They administered rats with single oral dose of hexachloronaphthalene isomers mixture (mainly 1,2,3,5,6,7- and 1,2,3,4,6,7-hexachloronaphthalene) and examined the retention in liver and adipose tissue. The L/A ratio determined by those authors was found to be remarkably high: 7.3 after 24 h and 1.1 after ten days [30].

Our study showed that 24 h after HxCN administration the retention in liver was highest (about 32% after p.o and 38% after i.p. administration). These results are in agreement with those obtained by Asplund et al. [30] who also observed the highest HxCN concentration in liver (about 35% of the administered dose) 24 h after its administration. Such a strong affinity to liver for tetra- and pentachlonaphthalene was not observed in our earlier experiment, in which the liver retention after a single dose of both compounds given to rats hardly reached several percent [48].

The present study also revealed that, while HxCN concentration in liver was significantly reduced five days after administration and the retention in this organ, regardless of the administration route, was comparable (16% after p.o. and 18% after i.p. administration), in adipose tissue the opposite effect was observed; after p.o. administration HxCN concentration gradually increased in adipose tissue, reaching the maximum level at the final time point (five days after administration). The observed retention in this tissue (about 26% of the received dose after five days) was comparable with that found in the experiment

performed by Asplund et al. [30]. However, they revealed that the level of HxCN concentration in adipose tissue reached the maximum value (about 35%) ten days after administration of the compound.

Constant increase in HxCN concentrations (regardless of administration route) was also observed in other organs (brain, sciatic nerve and adrenals), but due to their low absolute mass (mainly that of adrenals) they did not play a role in the total body balance. Nevertheless, it should be noted that HxCN concentrations in these organs in terms of w/w ratio were comparable with (or even higher than) those observed in kidneys or spleen. Therefore, the conjecture that not only liver but also the nervous system and adrenals may appear to be critical organs for HxCN cannot be ruled out.

In conclusion, hexachloronaphthalene has a slow turnover rate in the rat and, especially in the case of repeated exposure, may accumulate in the body, forming long-term storage compartments.

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