Bull. Environm. Contam. Toxicol. 26, 337-344 (1981)

Distribution and Elimination Routes of a Naphthenic Hydrocarbon (Dodecylcyclohexane) in Rainbow Trout *(Salmo gairdneri)*

J. P. Cravedi and J. Tulliez

Laboratoire de Recherches sur les Additifs Alimentaires, I.N.R.A., 180, chemin de Tournefeuille, 31300 Toulouse, France

Contamination of fish by hydrocarbons, whether it occurred directly via the water or indirectly via the food chain has been the object of many studies during the last decade. Unfortunately, because of the complexity of petroleum composition, there are many difficulties to draw a picture of what become petroleum components in aquatic organisms and to analyze the interactions of such compounds with fish biology.

The interest of laboratories has been focused on the most toxic components of crude oils, i.e. aromatic hydrocarbons, which are quantitatively of minor importance in petroleum while saturated hydrocarbons are even more abundant. The weak chemical reactivity of aliphatic hydrocarbons probably suggested a biochemical inertia that did not challenge lots of research on that subject. The studies in this field reported until now and reviewed by CORNER et al. (1976), concern essentially assimilation, transfer and discharge of the only n-alkanes, but there is a lack of information on the fate of cyclic alkanes in fish.

As assessed by TEAL (1977) naphthenic hydrocarbons are the least biologically active of the more mobile fractions of petroleum ; nevertheless the fate of these compounds are worth considering, because they constitute respectively 41 % and 19.2 % of light and heavy crude oils (BESTOUGEFF 1967).

The only work in which a cycloparaffin has been tested in fishes is reported by GRUGER et al. (1977) but it concerns the effect of a mixture of petroleum hydrocarbons on the hepatic aryl hydrocarbon hydroxylase activity, and does not investigate about distribution and disappearence of the individual compounds of the mixture.

However a few studies have been carried out with mammals: it has been shown that dodecylcyclohexane was highly absorbed (TULLIEZ and BORIES 1975a) and stored in adipose tissue (TULLIEZ and BORIES 1975b). Recently in vivo studies have been conducted to determine the routes and rates of biotransformation and elimination of dodecylcyclohexane in rat (TULLIEZ and BORIES 1979).

0007-4861/81/0026-0337 $01.60

This paper reports the results of our experiment in which ^{3}H-dodecylcyclohexane has been given per os to rainbow trout in order to evaluate the distribution and elimination routes of this cycloparaffin.

MATERIALS AND METHODS

Chemicals : Tritium-labelled dodecylcyclohexane (116 mCi/mM), was prepared by hydrogenation of ^{3}H-phenyldodecane like described by TULLIEZ and BORIES (1979). ^{3}H-dodecylcyclohexane was purified by thin-layer chromatography using silicagel plates and hexane as solvent. Chemical and radiochemical purities were checked by gas chromatography and radio-chromatography respectively.

Fish : Rainbow trouts (200-250 g) were obtained from I.N.R.A. hatchery at Donzacq (Landes, France) and held individually in aquarium (50 l) supplied with continuously flowing well water (12°C ± 2°C). Once a day, until 48 h prior to an experiment, animals were fed ad libitum, a commercial fish diet (Aqualim, France). All experiments were conducted during the months of February through April, under natural photoperiod.

Ingestion and excretion of hydrocarbon : Impregnation of a 0.4 g pellet with a 50 uCi ^{3}H dose was obtained after soaking in hexane. Two radio-labelled pellets were distributed to each fish, and after 30 mn, trouts were anesthetized in a 0.05 % (V/V) solution of 2-phenoxyethanol (Koch-light Lab. Ltd). A catheter inserted into the urinary duct to a depth of 2 cm, then ligated first to the urinary papilla and secondly to the base of the anal fin. The catheter was made with Silastic® tubing (602 - 155) on the outside of which were fixed two rings of Silastic® to help anchoring the sutures.

The fishes were placed in a PVC restraining pipe which prevented vertical and lateral body movements. This tube, pierced with numerous holes to facilitate water exchanges was located in another 50 liters aquarium supplied with water flow until complete wake up of the fish. Then, water flow was cut off and the water remaining in the tank was artificially aerated, using diffuser stones fed from a compressed air main.

Samples :

- Urine was collected at 3 h intervals by gravity flow into test tubes in a fraction collector, and radioactivity was determined by liquid scintillation counting (Intertechnique SL 32 apparatus). For the one week experiment, all samples were then pooled and extracted by hexane.

- Radioactivity in aquarium water was measured every 6 hours until 24 h and then, every day. After 1 week, an aliquote sample of water was extracted with hexane.

- Feces were taken out from the aquarium as fast as possible after their discharge.

Fishes were sacrificed by cervical dislocation 12h, 24h, 48h, and 1 week after the single dose ingestion of ^{3}H-dodecylcyclohexane. Analyses of organs and tissues have been carried out on trouts killed at 24 h and 1 week. Other fishes were cutted to bits, ground and homogenized with a Polytron homogenizer with cold water (2/1, w/v), after carefull removing of the gastro intestinal tract contents, in order to measure the radioactivity in trout carcass.

Aliquots of feces, tissues, organs and homogenized carcasses were extracted using Folch method. An aliquot of the resulting total lipids was saponified with ethanolic KOH, then the unsaponifiable fraction was extracted with hexane. Fatty acids were extracted with petroleum ether from the aqueous phase after acidification by HCl.

Total radioactivity of tissues, organs and feces was measured after combustion of aliquots (200-500 mg) in an oxidizer (Intertechnique Oxymat). Tritiated water in the different samples was estimated by comparing the results obtained by combustion of samples before and after lyophilisation.

Radio-gas liquid chromatography of the hexane extracts was carried out on a 1m. 3% SE30 column ; nine-tenth of the sample went through a proportionnal counter after hydrogenative cracking by a catalyser.

RESULTS

A metabolic balance was established (fig. 1) taking into account the radioactivity excreted in urine, feces and water, and the radioactivity remaining in the whole carcass. After a week, the percentage of ^{3}H was distributed as follows : feces 25 %, urine 19 %, aquarium water 30 %, whole carcass 26 %. The nature of labelled substances was not studied in detail. However, it has been shown that all the radioactivity detected in the feces was due to ^{3}H- dodecylcyclohexane. On the other hand, no hydrocarbon was found in urine. By this route, elimination reached its maximum level between 18 and 24 h after hydrocarbon administration. More than 15 % of the tritium ingested was excreted in urine within 4 days.

The radioactivity measured in the aquarium revealed a rapid elimination in the surrounding water : 8 % of the ingested radioactivity appeared in water after 12 h, when no feces have been yet excreted. Analysis of the hexane extract of water sample demonstrated a discharge of unchanged dodecylcyclohexane (between 2 and 6 % of ingested cycloparaffin).

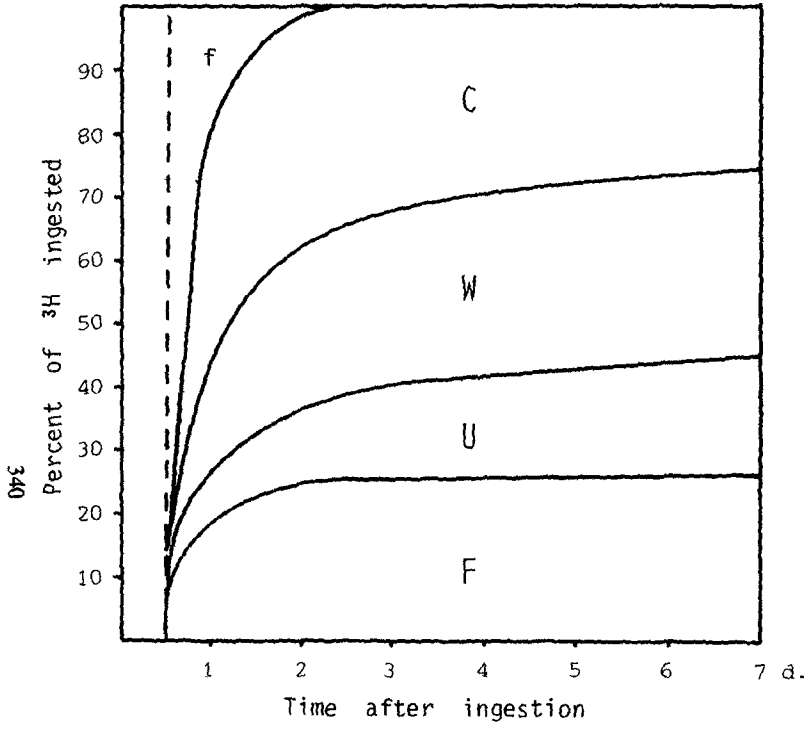

Fig. 1. Metabolic balance established after administration of one dose of dodecylcylcohexane

f = Labelled food in process of digestion ;
C = Carcass ; W = aquarium water ; U = Urine; F = Feces.

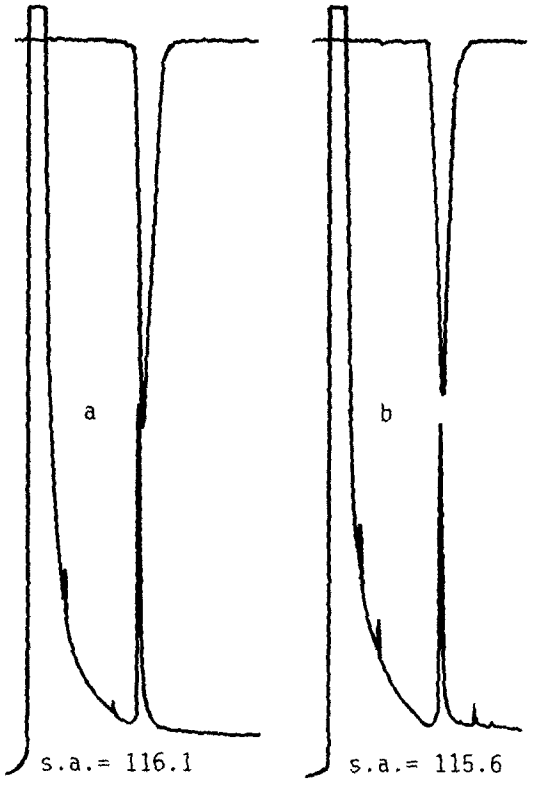

Fig. 2. Radio gas chromatography of hydrocarbon fed (a) and of hydrocarbon in carcass (b): upper trace= radioactivity; lower trace = mass; s.a.= specific activity (mCi/mM).

TABLE I

DISTRIBUTION OF ^{3}H IN TISSUES 24 H AND 1 WEEK AFTER INGESTION OF ^{3}H-DODECYLCYCLOHEXANE BY RAINBOW TROUT

	Tissue	Quantity	Total Radioactivity (% I)	^{3}H Recovered in unsaponifiable fraction		^{3}H Recovered in fatty acids		$\frac{^{3}H \text{ unsaponifiable}}{^{3}H \text{ fatty acids}}$
				% I	S.A.	% I	S.A.	
24 H	MUSCLE	128 g	15.5	13.4	0.1	1.5	0.01	10
	LIVER	2.1 g	1.7	0.9	0.43	0.7	0.33	1.3
	FAT	0.5 g	0.6	0.5	1.0	0.1	0.20	5
	SKIN	14.6 g	2.7	2.3	0.16	0.3	0.02	8
	KIDNEY	1.0 g	0.25	0.1	0.1	0.1	0.10	1
	GALL FLUID	500 µl	0.31	Und.	Und.	Und.	Und.	
1 WEEK	MUSCLE	126 g	9.5	8.2	0.07	1.1	0.01	7
	LIVER	2.2 g	0.6	0.3	0.18	0.3	0.14	1.29
	FAT	1.2 g	2.3	1.7	1.42	0.6	0.50	2.84
	SKIN	15.0 g	1.9	1.5	0.10	0.4	0.03	3.33
	KIDNEY	1.9 g	0.18	0.1	0.05	0.06	0.03	1.67
	GALL FLUID	600 µl	0.03	Und.	Und.	Und.	Und.	

% I = Percent of ingested ^{3}H
S.A. = Specific Activity µCi/g of fresh tissue
Und. = Undetermined

The carcass radioactivity increased rapidly until 24 h, levelled off between 24 and 48 h and then decreased daily of about 6 % . The activity recovered from the various fractions after Folch extraction showed the following distribution : 92 % in lipid extract, 6 % in aqueous fraction and 2 % in residual fraction.

The radio gas chromatogram presented on fig. 2b was obtained after injection of the unsaponifiable fraction. Retention time of the radioactive peak is identical to that of ^{3}H-dodecylcyclohexane (fig. 2a). More than 77 % of the carcass radioactivity was due to ^{3}H-dodecylcyclohexane. Specific activity of hydrocarbon retained in carcass was quite the same as specific activity of the hydrocarbon fed ; that proves the absence of tritium exchange.

The results of the measurements performed on tissues and organs taken after 1 and 7 days are noted in table I. It appears that total radioactivity in the various tissues drops slowly all along the experimental period, except in the fat where a great increase is observed. The highest level of radioactivity was found in adipose tissue (1.2 µCi/g after 24 h and 1.9 µCi/g after 1 week). ^{3}H-dodecylcyclohexane concentration in adipose tissue after one week was 1.42 times higher than after 1 day. The same ratio calculated for labelled fatty acid was 2.5. This suggests an important biotransformation of the cycloparaffin. The ratio presented in the last column showed a more rapid decrease of tritium in the unsaponifiable fraction than in fatty acids. Isolation of fatty acids from the carcass showed that 10 % of labelled compounds were present in this fraction.

It has been observed also that no accumulation of radioactivity occurred in gall bladder.

DISCUSSION

The metabolic balance pointed out that after one week, radioactivity was eliminated at analogous levels by the three possible routes for xenobiotic elimination by fish : feces, urine and surrounding water.

It is via the fecal excretion that the most important amount of unchanged dodecylcyclohexane was eliminated. Due to the absence of dodecylcyclohexane metabolites in feces, and to the fact that no unchanged hydrocarbon was detectable in the bile, it may be considered that labelled hydrocarbon eliminated in feces corresponds to the non-absorbed fraction of ingested cycloparaffin.

These preliminary results authorize an estimation of absorption level of 75 % ± 5 % of the ingested hydrocarbon. The high level of radioactivity (19 %) found in urine and the lack of unchanged hydrocarbon elimination via this route are the proof of the possibility of Rainbow trout to metabolize dodecylcyclohexane ; it is quite interesting to note that tritiated water in urine represents about 20 % of ^{3}H and the identification of the urinary metabolites would be of value to determine the enzymatic system involved in the cycloparaffin metabolization.

If elimination of ^{3}H in urine was important, the main part was observed in aquarium water. Further examination of the water samples showed that above all, tritiated water was the cause of the high level of radioactivity in aquarium.

This observation, as the presence of labelled fatty acids in the carcass suggest that the metabolism of dodecylcyclohexane may occur in the same way in fish andin mammals. Indeed TULLIEZ and PELERAN (1977) have pointed out in the rat that dodecylcyclohexane was ω-oxidized to cyclohexyldodecanoïc acid. Then, this unusual fatty acid was undergoing to classical β-oxidation (TULLIEZ and BORIES 1979) along the alkyl chain. If this mechanism occurs in trout, tritiated water might be attributed to this oxidative pathway that finally leads to its elimination through the gills.

A particular aspect of fish physiology highligthed by these experiments is their ability to reject ingested hydrocarbon as such in the surrounding water. This fact may be explained either by skin elimination or gills filtration. The rapid appearence of hydrocarbon in aquarium water, the high level of membrane exchange in gills and several papers concerning the excretion of lipophilic substances through the gills (BRODIE and MAICKEL 1962 ; MAREN et al. 1968 ; HUNN and ALLEN 1964) lead us to retain the latter hypothesis. In a study reported by CORNER et al. (1976), radioactivity was detected in aquarium water after distribution of ^{14}C-hexadecane to codling, but these authors did not identify the labelled substances excreted. To our knowledge, it is the first time that elimination of saturated a hydrocarbon via the gills is described.

If after one week a quarter of the ingested radioactivity remained in the whole carcass, it is interesting to compare the respective ^{3}H evolution between 24 h and 7 days in liver, carcass and adipose tissue ; while ^{3}H-level in liver decreased by two third, a four fold increase was observed in the fat and the corresponding decrease in the whole carcass was about 30 %. This phenomenon could result from the transfer of unoxidized hydrocarbon from liver to adipose tissue in which exogenous lipophilic substances are generally stored ; otherwise, it could be the result of oxidation in the liver of the cycloparaffin to

the corresponding fatty acid which is distributed in every tissue as indicated by the more rapid decrease of tritium in the unsaponifiable fraction of muscle fat and skin, when compared to ^{3}H evolution in the fatty acids.

The experiments discussed in the present paper gives evidence that Rainbow trout is able to absorb in a large scale a cycloparaffin distributed in the feed at a 0.02 % level ; intense metabolization occurs leading to metabolites which are excreted via the urines as well as through the gills. Work is in progress to identify the biotransformation products but also the possible physiological effects of a long term diet contamination.

AKNOWLEDGEMENTS

This study was supported in part by grants from Commission of European Communities, under Contrat n° 188-77 1 ENV F.

REFERENCES

BESTOUGEFF M.A. : in Fundamental aspects of geochemistry. Chap. 3, Amsterdam : Elsevier (1967).

BRODIE B.B. and R.P. MAICKEL : Proc. 1 st. Intern. Pharmacol. Meeting : Mode of Action of Drugs. Vol. 6. p. 299. Oxford Pergamon Press (1962).

CORNER E.D.S., R.P. HARRIS, K.J. WHITTLE and P.R. MACKIE : In Effects of pollutants on aquatic organisms. Vol. 2. p. 71. London : Cambridge University Press (1976).

GRUGER E.H. Jr, M.M. WEKELL and P.A. ROBISCH : in Fate and effects of petroleum hydrocarbons in marine organisms and ecosystems, p. 323. Oxford : Pergamon Press (1977).

HUNN J.B. and J.L. ALLEN : Ann. Rev. Pharmacol. 14, 47 (1974).

MAREN T.H., R. EMBRY and L.E. BRODER : Comp. Biochem. Physiol. 26, 853 (1968).

TULLIEZ J. and G. BORIES : Ann. Nutr. Alim. (Paris) 29, 201 (1975 a).

TULLIEZ J. and G. BORIES : Ann. Nutr. Alim. (Paris) 29, 213 (1975 b).

TULLIEZ J. and G. BORIES : Lipids, 14, 292 (1979)

TULLIEZ J. and J.C. PELERAN : FEBS Lett. 75 (1), 120 (1977).