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Accumulation, Distribution and Depuration in Trout of Naphthenic and Isoprenoid Hydrocarbons (Dodecylcyclohexane and Pristane)

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The biological effects in the aquatic environment caused by hydrocarbon pollution have been largely investigated. In order to determine the impact of such complex pollutants on aquatic species, numerous studies have been documented on the uptake and depuration of n-alkanes and aromatic hydrocarbons as reviewed by LEE (1977), but little attention has been given to branched and cyclic paraffins. Nevertheless, these compounds are present in all crude oils in large concentration (POSTHUMA 1977), and it was of interest to assess the accumulation and the distribution of such hydrocarbons in fish tissues and carcass and to determine the rate and conditions of their discharge.

Pristane (2, 6, 10, 14-tetramethylpentadecane), which is a major alkane component in the aquatic ecosystem (BLUMER et al. 1964 ; WHITTLE et al. 1974) and particularly in several freshwater and marine fish (ACKMAN 1971 ; WHITTLE et al. 1977) has been taken as representative of branched alkanes. Dodecylcyclohexane, identified as a component of crude oils (LAWLER et al. 1978) and used at several occasions as a naphthenic hydrocarbon for metabolic studies in rat (TULLIEZ and BORIES 1975a, 1975b, 1979), was the model adopted for cycloparaffins.

The major features of the data available on the biological transfer processes of these two contaminants in animal species are the efficient absorption and retention of these compouds in rat (TULLIEZ and BORIES 1975a), the preferential fixation in adipose tissue in rat (TULLIEZ and BORIES 1975b) and chicken (TULLIEZ 1975) and the slow mobilization of the stored amount (TULLIEZ and BORIES 1975b).

In a recent investigation (CRAVEDI and TULLIEZ 1981) we found an analogous trend in rainbow trout after a single ingestion of ^{3}H-dodecylcyclohexane. However no investigations had been performed with pristane and it was of interest to have long term exposures, to evaluate the possible effects on fish.

The purpose of this work was to determine the rate of dodecylcyclohexane and pristane accumulation, the uptake level, the ability of tissues and organs to accumulate these hydrocarbons, and the possibility for the trout to discharge the stored hydrocarbons, when fed an hydrocarbon-free diet or kept in starvation.

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MATERIALS AND METHODS

Experimental procedures.

Analytical grade (99 % min.) hydrocarbons used were obtained from Koch an Light Lab. Purity of these compounds was tested by gas liquid chromatography (G.L.C.). Fish exposure to hydrocarbons was conducted in the I.N.R.A. pisciculture at Donzacq, Landes (France).

Rainbow trout (*Salmo gairdneri R.*) of uniform size, obtained at the hatchery of Les Athas (64, France) were randomly divided in three groups of 800 fish and placed in water ponds supplied with freshwater at constant temperature (17 ± 1°C). The pH of the water was constant at 7.1. Initial mean weight of the fish was 13.0 ± 0.9 g.

Trial I : Accumulation period.

Fish were fed pellets, distributed *ad libitum* four times a day, until a final weight of 170 g was attained. The control group received pellets, the composition of which was described by FAUCONNEAU and LUQUET (1979). Experimental groups received the same pelleted feed where 1 % hydrocarbon (dodecylcyclohexane or pristane) was instead of the same amount of soy oil. Mortality was recorded daily. Randomly sampled lots of 100 trout were weighed every week during the first month and monthly thereafter. In each group, 20 fish were sampled at 1, 2, and 4 weeks and monthly thereafter. Ten additional fish were removed when they reached 170 g. The fish were sampled 24 h after the last meal, killed by electrocution, weighed and stored at -20°C.

Trial II : Depuration period.

The remaining trout in each group were equally divided in two groups : the first received control diet *ad libitum* for a 2 month period ; the second was maintained in total fast for the same period. During this depuration period, 20 trout were removed from each group after 1 and 2 months. The sampling procedure was that mentioned above.

Methods of analysis

Each sample of 20 trout was randomly divided in 5 groups of 4 fish on which the amount of stored hydrocarbons and the percent of dry matter in the carcass were measured.

Hydrocarbon concentration was determined in organs (liver, kidney, spleen) and tissues (fat, muscle, skin, intestine) sampled from the 10 trout sacrificed at the end of the accumulation period. All the lipidic adhesions were removed from the intestine before analysis. Perigastric adipose tissue was used as the fat sample. Whole trout were minced, ground and homogenized with cold water (2:1 w/v) after carefull removal of the gastrointestinal contents. The analytical procedure used is schematically presented in Figure 1.

All solvents used were redistilled and tested by G.L.C. after concentration. Hydrocarbons were extracted as described in a previous report (CRAVEDI and TULLIEZ 1981). Before extraction, an internal standard was added to the sample : eicosane for pristane measurements and tetracosane for dodecylcyclohexane and control trials. Hydrocarbons were separated using a Packard 7300 gas chromatograph equipped with a flame ionization detector and a 1 m x 2.5 mm i.d. glass column packed with 3 % Dexsil 300 on chromosorb W AW DMCS 80/100. The nitrogen carrier gas flow rate was maintained at 20 ml/min. The column temperature was programmed from 120°C to 300°C at 10°C/min and maintained 2 min at the initial temperature and 3 min at 300°C. The Student's t test was used to compare results from the different lots.

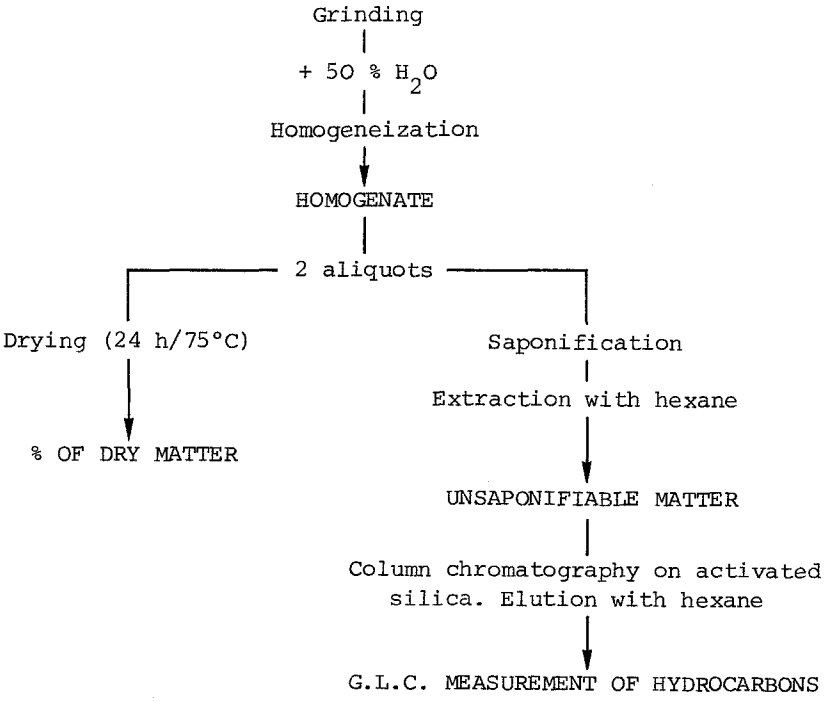

Fig. 1 - Scheme of isolation and identification of saturated hydrocarbons.

RESULTS

The mortalities in control and experimental groups were in the same range, and the level was that normally observed in the pisciculture.

TABLE 1

ACCUMULATION OF DODECYLCYCLOHEXANE AND PRISTANE IN TROUTS

RECEIVING HYDROCARBON CONTAMINATED DIETS

(whole carcass measurements)

Time fed (weeks)	Mean weight fish (g)			Hydrocarbon concentration[b] (ppm per wet weight)		
	C	D	P	C[c]	D	P
1	15.1	14.5	14.6	0.7 ± 0.1	166.0 ± 39.7	94.4 ± 31.6
2	15.9	15.8	15.0	1.1 ± 0.2	238.5 ± 30.8	102.2 ± 33.1
4	21.9	17.2	19.3	1.0 ± 0.3	308.3 ± 50.3	168.0 ± 33.1
12	45.2	32.8	32.6	0.8 ± 0.2	643.2 ± 71.7	370.9 ± 103.1
16	86.3	51.4	46.2	und.	555.5 ± 120.8	und.
20	115.5	69.5	64.5	0.7 ± 0.2	571.9 ± 68.9	391.4 ± 44.9
24	150.4	76.0	83.7	0.9 ± 0.1	562.4 ± 111.9	546.0 ± 157.8
28		83.0	104.0		602.1 ± 162.5	574.0 ± 190.3
End[a]	176.3	170.0	188.3	1.3 ± 0.4	718.1 ± 86.2	556.11± 129.4

C = control group ; D = dodecylcyclohexane ; P = pristane group ; und. = undertermined

a = end of accumulation period occurred after 26 weeks for control group, after 38 weeks for dodecylcyclohexane group and after 44 weeks for pristane group.

b = values are means ± standard deviations.

c = no dodecylcyclohexane was detected, the values concerned the amount of pristane.

Accumulation period

Both hydrocarbons caused an apparent reduction of growth of fish : control group required 6 months to reach 170 g, whereas dodecylcyclohexane and pristane groups needed respectively 9 months and 10 months (Table 1).

No significant difference in dry weights appeared between contaminated trout and control group at $p < 0.05$; the mean value for most samples was 25-28 mg/g.

Hydrocarbons concentration in trouts increased during the first months of exposure and reached a steady state after about 3 months for dodecylcyclohexane group and 6 months for pristane group. At this time, concentrations were in the 550-600 ppm range, corresponding to a quantity of 122 mg per fish of dodecylcyclohexane and 105 mg per fish of pristane at the end of the accumulation period, i.e. respectively 3.6 and 2.5 % of the quantity theorically ingested. The weak concentration of pristane observed in control group issues from the trace of this hydrocarbon in the herring meal which constituted 35 % of the diet.

The results of the measurements performed on tissues and organs taken after 9 months (for dodecylcyclohexane) and 10 months (for pristane) are noted in Table 2. The major feature of these results was the recovery in the perigastric adipose tissue of respectively 12.6 % (dodecylcyclohexane group) and 8 % (pristane group) of the hydrocarbons retained in the total carcass, whereas this tissue represented only 2 % of the body weight. No hydrocarbons were stored in the liver of contaminated trout.

Samples	Dodecylcyclohexane	Pristane
Carcass	718 ± 86	556 ± 129
Perigastric adipose tissue	11276 ± 884	6182 ± 1613
Skin	604 ± 157	426 ± 81
Intestine	457 ± 25	141 ± 20
Muscle	299 ± 62	239 ± 37
Spleen	164 ± 15	283 ± 28
Kidney	130 ± 7	163 ± 15
Liver	15 ± 8	51 ± 25

Table 2 - Distribution of dodecylcyclohexane and pristane in trout . Values are means ± standard deviation, reported as µg/g.

TABLE 3 - DEPURATION OF PRISTANE AND DODECYLCYCLOHEXANE IN TROUT

		Starved		Fed	
		1 month	2 months	1 month	2 months
Mean	C	154.3	150.1	211.1	240.0
weight	D	152.5	160.2	202.8	250.0
Fish (g)	P	129.3	105.0	183.0	236.1
Dry	C	26.99 ± 1.09[b]	26.54 ± 1.66[b]	30.05 ± 1.31	30.63 ± 1.09
matter[a]	D	26.66 ± 0.95[b]	25.29 ± 1.18[b]	30.56 ± 1.35	29.11 ± 1.83
(%)	P	22.90 ± 1.95[b]	26.56 ± 1.44[c]	28.0 ± 0.18	29.25 ± 1.34
Hydrocarbon	C[d]	1.2 ± 0.2	4.5 ± 1.1	2.5 ± 0.9	1.5 ± 0.6
concentration[a]	D	633.1 ± 187.2	802.2 ± 173.9	484.6 ± 115.2	276.4 ± 104.1
(ppm)	P	420.7 ± 74.4	564.8 ± 54.9	397.2 ± 115.5	251.1 ± 85.1

C = control group ; D = dodecylcyclohexane group ; P = pristane group.

a = values are means ± standard deviations
b = significantly lower than fed group, $P < 0.01$
c = significantly lower than fed group, $P < 0.05$
d = no dodecylcyclohexane was detected, the values represent the amount of pristane.

Depuration period

The effects of fasting or feeding control diet, on hydrocarbon concentrations of contaminated trout are presented in Table 3. Comparison of the body weight in the various groups during starvation was difficult because the lack of homogeneity of fish sizes involved problems of non representative sampling. However, it is clear that contaminated trout recovered normal growth rate when the hydrocarbon-free diet was resumed. The dry matter measurement exhibits the usual increase of water content in starved fish.

On average, after two months experiment, residues in the starved trout amounted to the totality of the level reached at the end of the accumulation time. The animals receiving a hydrocarbon-free diet during the same period only retained 60 % of the quantity of hydrocarbon stored.

DISCUSSION

Chronic accumulation of dodecylcyclohexane and pristane in trout was studied and incorporation of these saturated hydrocarbons occurred to the greatest extent in adipose tissue. This is in agreement with our previous report on the distribution of ^{3}H-dodecylcyclohexane in trout after a single administration of this cyclo-paraffin (CRAVEDI and TULLIEZ 1981) and with the data given by TULLIEZ and BORIES in the rat (1975a). Dodecylcyclohexane and pristane were detected in every tissue examined. The low level of hydrocarbon detected in the liver suggests that pristane and dodecylcyclohexane were metabolized or removed from the liver rapidly after the meal and before the fish were sacrificed. This hypothesis has been expressed by others concerning saturated hydrocarbons distribution in cod (HARDY et al. 1974) and mallard ducks (LAWLER et al. 1978)

The concentration of dodecylcyclohexane in trout carcass at saturation was 2 to 2.5 orders of magnitude higher than in rat (TULLIEZ and BORIES 1975b). No similar data were obtained when pristane storage was concerned. This experiment points out that accumulation of this hydrocarbon in trout occurred 2 times more slowly, and the final saturation level was lower than for dodecylcyclohexane. This confirms the observation of CLEMENT et al. (1980) that within the aliphatic fraction of oil accumulated by clams, the cyclic compounds were generally retained to a greater extent than branched chains.

Starvation seems to be an adverse situation for depuration of hydrocarbons in trout. Despite 2 months starvation, the amounts of hydrocarbons stored in the carcass did not decrease, in contrast with the results observed in mammals by TULLIEZ and BORIES (1975b). These authors noticed that the rate of mobilization of stored dodecylcyclohexane and eicosane by animals fed and energy-restricted diet was twice that of those receiving control diet *ad libitum*.

Nevertheless, our results are in agreement with the data of BLUMER et al. (1964) who observed that pristane content was unaltered or increased during starvation in the copepod *Calanus hyperboreus*. These comparisons point out that in aquatic organisms, the mobilization of such foreign compounds occurred very slowly, especially during starvation. This might be partly due to a slow lipid utilization resulting from a low metabolic rate during starvation. However, the catabolism of the major lipids deposit do not involve the elimination of lipophilic xenobiotics such as alkanes (TULLIEZ and BORIES 1975b) and may reflect the necessity of biotransformation of the hydrocarbon before its excretion (CRAVEDI and TULLIEZ 1981).

The lack of enhanced mortality in trout fed dodecylcyclohexane and pristane for 38 and 44 weeks, respectively, shows that these compounds were non toxic at this level of contamination. However, the reduction of growth noted and recently observed by LUQUET et al. (unpublished data) was a phenomenon unknown for the other animal species investigated:rats (TULLIEZ and BORIES 1975b) and chickens (TULLIEZ 1975). The return to a normal growth rate indicates that the inhibition observed was not an irreversible process. Further research is necessary to explain this phenomenon.

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