Bull. Environ. Contam. Toxicol. (1997) 59:543-547

Bulletin of Environmental Contamination and Toxicology

Levels of Aliphatic Hydrocarbons in Viscera of Wolves (*Canis lupus*, L) in Galicia (N.W. Spain)

S. T. Carril González-Barros,[1] M. E. Alvarez-Piñeiro,[2] J. Simal-Lozano,[1] M. A. Lage-Yusty[1,2]

[1]Department of Analytical Chemistry, Nutrition of Bromatology, Area of Nutrition and Bromatology, Faculty of Pharmacy, University of Santiago de Compostela, Campus s/n, 15706 Santiago de Compostela (La Coruña), Spain
[2]Laboratory of Bromatology, Institute of Investigation and Food Analysis, Faculty of Pharmacy, University of Santiago de Compostela, Campus s/n, 15706 Santiago de Compostela (La Coruña), Spain

Received: 29 April 1997/Accepted: 11 June 1997

Accidental oil spills and the careless disposal of petroleum products can seriously damage the biosphere. Among the earliest manifestations of this damage is the partial destruction of flora and fauna (Korringa 1982). In the latter case, contamination occurs both directly and, by virtue of the higher position of animals in the food chain, indirectly through diet. Frequently, this results in the accumulation of hydrocarbons in tissues such as liver, brain and muscle (Fraser 1993; Macia-Zamora 1993).

Most studies of the bioaccumulation of n-alkanes in animal tissues have dealt with marine life-forms such as molluscs, crustaceans and fish (Hermida et al. 1995; Snedaker et al. 1995; Carril et al. 1996a). In view of this, and the increasing importance of environmental monitoring in general, it is of interest to extend this type of study to non-marine animals. Of particular interest are mammals at the upper end of the food chain, firstly as bioindicators of terrestrial contamination, and secondly because this category includes several species in danger of extinction.

In the present work, GC-FID was used to determine aliphatic hydrocarbons in viscera of male and female wolves of various ages, from the Autonomous Community of Galicia (N.W. Spain).

MATERIALS AND METHODS

Fifty-six samples of wolves' viscera (12 of liver, 12 of suprarenal capsule, 11 of kidney, 11 of spleen and 10 of muscle) were supplied, following classification, by the Parasitology Laboratory of the Institute of Food Research and Analysis of the University of Santiago de Compostela. The samples came from seven males and five females aged between 6 months and 3 years. Samples were small (minimum mass, 0.5033 g.).

Reagents were n-hexane, dichloromethane and anhydrous sodium sulfate, all from Merck; and Sep-Pak® Silica Plus cartridges, from Millipore. Pristane and C_{18}, C_{24}, C_{32} and C_{36} hydrocarbon standards were from Alltech; and C_{19}, C_{20} and C_{22} standards were from Chem Service.

Correspondence to: M. A. Lage-Yusty

A Telstar freeze-dryer. A Perkin-Elmer 8500 gas chromatograph equipped with a Perkin-Elmer AS 8300 autosampler, a Sugelabor SGL-5 capillary column (0.1 µm of 5% diphenylmethylsilicone; 25 m x 0.25 mm i.d.), and a flame-ionization detector connected via Labnet to a Spectra-Physics SP4270 integrator and an Olivetti PRO 16SX PC for data acquisition and processing.

Samples were analysed by a similar method to that described by Carril et al., 1996b and 1996c. Briefly, each viscera sample was lyophilized at 0.1 mmHg vacuum and between -20 and +25° C for 24-48 h, and 0.5 g of the lyophilizate was extracted with 1:1 n-hexane/dichloromethane in a Soxhlet for 8 h. The organic solvent was evaporated under a nitrogen stream, and the soluble extract was oven-dried at 100° C for 1 h 30 min. and reweighed. This fatty extract was then redissolved in n-hexane and cleaned up on a Sep-Pak® Silica Plus cartridge; the fraction eluting in 10 mL of n-hexane was concentrated to 1 mL and 1 µL was chromatographed on the above system.

The hydrocarbons in the samples were identified by comparing the sample chromatograms with those of standards. Quantification used the external standard method. Recoveries were satisfactory averaging 87.8 5.6 %. Method precision and reproducibility were both good.

RESULTS AND DISCUSSION

For each type of viscera, Table 1 lists the mean soluble fat content (%), the content in each of the hydrocarbons (mg/kg of dry fat), and the total hydrocarbon content, which was calculated as the sum of the areas of all hydrocarbon peaks multiplied by the response factor for C_{18}. The highest soluble fat content was 89.78% for suprarenal capsule, followed by 19.26% for muscle; the latter value is rather high, and also had a high standard deviation (19.26 mg/kg), suggesting that not all the fat had been separated from the muscle samples.

As regards the individual hydrocarbons, C_{36} was only detected in suprarenal capsule, and C_{32} was only detected in suprarenal capsule and muscle. The remaining hydrocarbons were detected in all the tissues except kidney, for which only the total hydrocarbon content was estimated. Levels of individual hydrocarbons varied widely, but were generally highest for C_{28}, C_{22}, C_{20}, and C_{18}, which were most abundant in muscle (22.09, 18.50, 9.70 and 11.35 mg/kg, respectively). Muscle also had the highest total hydrocarbon content (1665.67 mg/kg), closely followed by the much fattier suprarenal capsule (1411.29 mg/kg).

Analysis of variance for the individual hydrocarbon contents indicated that pristane and C_{19} contents were significantly higher ($p \leq 0.05$) in muscle than in the other viscera; that C_{28} content was significantly higher ($p \leq$

0.05) in muscle than in all the other viscera except suprarenal capsule; and that C_{22} content was significantly higher ($p \leq 0.05$) in muscle than in kidney.

Table 1. Mean contents of the wolves' viscera in soluble fat (%) and individual (mg/kg of dry fat) and total (mg of C_{18} equivalents/kg dry fat) aliphatic hydrocarbons.

	SPLEEN	LIVER	MUSCLE	KIDNEY	SUPRARENAL
FAT (%)	9.31	10.48	19.26	10.07	89.78
PRISTANE	ND*	0.48	5.03	ND	1.96
C_{18}	6.32	4.72	11.35	ND	3.78
C_{19}	ND	0.94	6.57	ND	3.13
C_{20}	6.22	5.48	9.70	ND	0.52
C_{22}	10.57	15.49	18.50	ND	1.10
C_{24}	5.85	7.95	12.78	ND	7.62
C_{28}	0.85	2.50	22.09	ND	15.53
C_{32}	ND	ND	12.17	ND	6.32
C_{36}	ND	ND	ND	ND	1.04
TOTALS	702.08	553.98	1665.67	557.85	1411.29

*ND= not detecteable

Table 2 shows the hydrocarbon contents of the viscera grouped according to the wolves' sex and approximate age (adults and subadults, the latter aged ≤ 18 months). These data show that pristane and C_{18}, C_{19}, C_{20}, C_{22} and C_{24} contents, and also total hydrocarbon contents, were lower in

subadults than in adults; that C_{28} and C_{32} contents were higher in subadults than in adults; and that C_{36} content was similar in adults and subadults. As regards sex, pristane and C_{18}, C_{19}, C_{20} and C_{22} contents were higher in males than in females, whereas the remaining hydrocarbon contents varied little between the sexes. Total hydrocarbon content was also higher in males than in females,

Table 2. Mean individual (mg/kg dry fat)and total (mg of C_{18} equivalents/kg dry fat) aliphatic hydrocarbon contents grouped according to wolf age and sex.

	AGE		SEX	
	ADULT	SUBADULT	MALE	FEMALE
FAT (%)	26.91	37.92	28.78	28.66
PRISTANE	1.55	0.92	1.62	1.09
C18	8.81	3.82	6.63	2.53
C19	3.16	1.28	2.30	1.61
C20	6.89	5.52	4.85	3.21
C22	15.23	11.51	10.67	6.03
C24	10.94	7.11	6.82	6.68
C28	8.31	11.97	7.67	8.48
C32	1.65	6.33	3.66	3.31
C36	0.18	0.20	0.10	0.42
TOTALS	928.76	800.83	966.72	964.95

In the absence of data for the aliphatic hydrocarbon contents of other mammals, these results for wolves were compared with those for various marine samples (Al-Saad 1990; Serrazaneti et al. 1991; Hermida et al. 1994; Quintero and Diaz 1994; Aboul-Kassim and Simoneit 1995; Snedaker 1995). Generally, wolves' viscera had lower total aliphatic hydrocarbon contents than these marine samples, but had higher contents in the individual hydrocarbons determined in this work.

Acknowledgements

We thank the staff of the Parasitology Laboratory of the Institute of Food Research and Analysis of the University of Santiago de Compostela, especially Dr. Manuel Sanmartin Durán, whose collaboration made this work possible.

REFERENCES

Aboul-Kassim TAT, Simoneit BRT (1995) Petroleum hydrocarbon finger printing and sediments transport assessed by molecular biomarker and multivariate statistical analyses in the Eastern harbour of Alexandria, Egypt. Mar Poll Bull 30:63-73

Al-Saad HT (1990) Distribution and sources of aliphatic hydrocarbons in fish from the Arabian Gulf. Mar Poll Bull 21:155-7

Carril González-Barros, S.T., Alvarez Piñeiro, M.E., Simal Lozano, J. & Lage Yusty, M.A. (1996a). Pollution by aliphatic hydrocarbons on the coast of La Coruna (NW Spain) after the Aegean Sea oil spill. Toxicol. Environ. Chem. (In press)

Carril González-Barros ST, Alvarez Piñeiro ME, Simal Lozano J, Lage Yusty MA (1996b) Comparación de disolventes para la extracción de materias grasas en cerdo. Grasas y Aceites 47 (In press)

Carril González-Barros ST, Alvarez Piñeiro ME, Simal Lozano J, Lage Yusty MA (1996c) Simultaneous determination of aliphatic hydrocarbons, PCBs and PCTs in pork liver by gas chromatography. Chromatographia 43:398-400

Fraser CM (1993) El manual Merck de veterinaria. 4th. Océano/Centrum, Barcelona

Hermida Ameijeiras A, Simal Gándara J, Lopez Hernández J, Simal Lozano J (1994) Aliphatic hydrocarbon levels in farmed and free-living mussels from Galicia (NW Spain). Mar Pollut Bull,28:178-81

Hermida Ameijeiras A, Simal Gandára J, Lopez Hernández J, Simal Lozano J (1995) Evaluation of performance of a multiresidue gas chromatographic method for the determination of aliphatic hydrocarbons in mussels of Galicia (NW Spain). Wat Res 9:2118-124

Korringa K (1982) El océano como receptor último de los productos finales del metabolismo del continente. Contaminación de los océanos: situación, consecuencias y previsiones para el futuro. Ecologia y protección de la naturaleza. Conclusiones internacionales. Blume, Barcelona

Macias-Zamora JV (1996) Distribution of hydrocarbons in recent marine sediments of the Coast of Baja California. Environ Pollut 92:45-53

Quintero S, Díaz C (1994) Aliphatic hydrocarbons in fish from the Canary Islands. Mar Poll Bull 28:44-9

Serrazanetti GP, Conte LS, Carpene E, Bergami C, Fonda-Unani S (1990) Distribution of aliphatic hydrocarbons in plankton of Adriatic Sea open waters. Chemosphere 23:925-38

Snedaker SC, Glynn PW, Rumbold DG, Corcoran EF.(1995) Distribution of n-alkanes in marine samples from Southeast Florida. Mar Pollut Bull 30:83-9