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ORGANOCHLORINE LEVELS IN LIVER OF WILD GRASS CUTTER (*Thyronomys swinderianus*) USING GAS CHROMATOGRAPHY

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ABSTRACT

Preliminary investigations on the concentrations of organochlorine compounds in the liver of wild grass cutter (*Thyronomys swinderianus*) from Ore, Ondo State, Nigeria is reported. Eighteen liver samples were analysed for 18 chloropesticides and related residues, as well as PCBs. Fourteen of the 18 compounds and PCBs were detected in all samples but the concentrations were generally low (< 12 µg/kg, wet weight). p,p'DDD, p,p'DDE, and p,p' DDT were the dominant pesticides observed. Low chlorinated biphenyls (2,4,4' tri, 2,2',4,5'- 2,2',5,5'-tetrachlorobiphenyls) were detected in 40 % of the samples. High chlorinated biphenyls (2,2',3,4,4',5'-, 2,2',4,4',5,5'-hexa-2,2'3,4,4',5,5',-heptachlorobiphenyl) were detected in each sample. Hexachlorobenzene (HCB) was detected in 86% of the samples while lindane and isomers 52%, p,p'DDE 100%, p,p'DDD 100%, p,p'DDT 100%, and dieldrin 96%. The levels of ΣDDT were higher than ΣPCB showing more anthropogenic impact of agriculture compared to the industry. However, the PCBs and pesticide levels were below maximum WHO permissible levels. The results show that wild grass cutter (*Thyronomys swinderianus*) is a useful bio-indicator of environmental contamination with chlorinated pesticides and PCBs. Residue analyses of liver samples of *Thyronomys swinderianus* should be integrated in future environmental monitoring programs.

Key Words: DDT, Polychlorobiphenyls, WHO, Ore, Nigeria

INTRODUCTION

Polychlorinated biphenyls (PCBs) and organochlorine pesticides are semi-volatile organo-chlorine hydrocarbons that have penetrated all ecosystems via long range transport (particularly in air), including remote areas such as Arctic and Antarctic (Risebrough *et al.*, 1968, Muir, *et al.*, 1988). These classes of hydrocarbon compounds are persistent, lipophilic and strongly hydrophobic chemicals with high bio-concentration potentials and have been linked to adverse effects in organisms at different trophic levels (Tanabe, 1988). Recent investigation of pathways and transfers of these compounds in soils have highlighted their transfer to livestock, wildlife and humans (Wild & Jones, 1992). These groups of environmental contaminants have been subjected to detailed chemical analyses, toxicological and risk assessments in many environmental matrices because of their stable properties and ubiquitous distribution (Jones *et al.*, 1991). Only a fraction of PCB congeners are found in environmental samples (McFarland & Clarke, 1989). It is well established that mechanisms exist for chemical modification and excretion of DDT in mammals. Initial degradation of DDT (1,1,1-Trichloro-2,2-bis(4 chlorophenyl) ethane may proceed either by substitution of one chlorine by a hydrogen atom yielding saturated, 1-dichloro-2,2-*bis*(4-chlorophenyl)ethane (p,p′-DDD) or by dehydrochlorination yielding the unsaturated (1-chloro-2,2-*bis*(4-chlorophenyl)ethylene) p,p′-DDE (Kaushik, 1991, Guenzi & Beard, 1976, Mitra & Raglu, 1986). p,p′-DDD, readily degrades further

through a series of intermediates to the excretable p,p′-DDA, (bis[p-chlorophenyl]acetic acid) which is rarely found as a stored metabolite in environmental samples. By contrast, p,p′-DDE apparently does not undergo further breakdown. This stability accounts for higher animal tissue store of p,p′-DDE than p,p′-DDD in environmental samples. When PCBs and DDT and its metabolites are analysed, other chlorinated pesticides like, dieldrin, aldrin, endrin, lindane and isomers, hexachlorobenzene (HCB) are also detected (Unyimadu, 2001). Data on the half-life (t½ d) in soil of organochlorine pesticides abound in literature, however the value depends on the quantity in kg/ha applied and also the mode of application (Wandiga & Natwaluma, 1984). The half- life of the different pesticides are highlighted in Table 1.

It is an acceptable fact that wildlife are useful bio-indicators of environmental contamination, serving as effective and simple indicators of pollution mobilization and consequential risk. Wildlife population dynamics have been affected by high dietary exposure to PCBs and DDT which impact upon the size, maturation and structural integrity of the sex organs (Batty, *et al.,* 1990). The levels of organochlorine pesticides and polychlorinated biphenyls in the tissues and organs of wildlife reflect the degree of contamination of the environment by these compounds (Drescher-Kaden *et al.,* 1978). Levels of these compounds have been determined in fish (Unyimadu, 2001,Osibanjo, *et al.,* 1994, Fayomi, 1987, Amakwe, 1984) and in mammals (Holm, 1993). The objective of this study was to provide baseline data on important organochlorine contaminants in the liver of a game animal, *Thyronomys swinderianus,* compare the concentrations with maximum levels permissible by law (WHO, 1976, 1989) and determine the origin of contamination..

MATERIALS AND METHODS

Sampling

The grass cutters were collected from the wild by traps between Okitipupa and Ore, Ondo State, Nigeria. It is an animal that is found in the forest and savanna regions of Nigeria. It belongs to the rodent family. In addition to grass, they eat maize, cassava sorghum, millet, ground nut and palm kernel. The palm kernels are from the palm plantations in Okitipupa where mechanized agriculture with extensive use of agro-chemicals was the practice. The other crops were produced by subsistence farming with no known use of agro-chemicals. The Doe (female) have short gestation period, (154 days), suckling period (21-56 days) and produce two litters per year. The Does can bear litters while six months old. The size of the litter is minimum of six and maximum of eleven fries. The age and sex of the Doe and Buck (male) selected for dissection could not be determined but each weighed approximately 4 kg.

Experimental

Eighteen liver samples were collected in May 2001, and analysed in August 2001. Individual liver samples weighted between 135.6 to 191.8 g. About 30g of tissue was collected from each liver sample washed with distilled water, packed in aluminium foil and preserved by freezing until analysis. Full experimental details are given elsewhere (Weichbrodt, *et al..,* 2000,Vetter, *et al..,* 1995, 1998, Steinwandter, *et al..,* 1978). About 10.0 g of fresh liver fillet was homogenized in a mortar with 10 g of Na_2SO_4. The homogenate was carefully transferred to the extraction chamber of a Focused Open Vessel Microwave Assisted Extractor (FOV-MAE). The extraction of the liver homogenate was performed twice after the addition of an internal standard, perdeuterated α-HCH (α-PDHCH) with 60 ml ethyl acetate-cyclohexane (1:1, v/v). After volume adjustment to exactly 10 ml, the extracts were filtered through 0.45 µm membrane filters. 1 ml was retained for gravimetric lipid determination. The remaining sample (9 ml) was automatically introduced into the 5 ml sample loop of the Gel Permeation Chromatography (GPC) system. GPC was also performed with ethyl acetate cyclohexane (1:1, v/v) as the solvent. Adsorption chromatography was performed using activated silica gel (G60, Merck, Darmstadt, Germany) which was deactivated with 30% water (w/w) by shaking for 30 min. A 3 g portion of the deactivated silica gel was slurry packed into a glass column (1.0 cm i.d.) and covered with Na_2SO_4. The isooctane extract of the sample was placed on the silica gel column and the column was eluted with 60 ml hexane. The eluate was concentrated by rotary evaporation and by a nitrogen stream to 0.5 ml. Aliquots were analysed by GC-ECD for the determination of PCBs and organochlorine pesticides. The GC separation was performed on a Hewlett-Packard 5890 series II gas chromatograph equipped with two capillary columns and two ^{63}Ni electron capture detectors (ECD). The injector and detector temperatures were set at 2200°C (splitless) and 300 °C respectively. Helium was used as carrier gas at a head pressure of 0.9 bar and nitrogen was used as make up gas. CP-Sil 8 and CP-Sil 2 were used as stationary phase. The later stationary phase is less than half as polar as 100% dimethylpolysiloxane (Vetter, *et al.,* 1998). Both columns were of 50 m length, 0.25 mm internal diameter, and 0.25 µm film thickness. The GC oven temperature started at 60 °C (1.5 min), then the temperature was ramped at 40 °C/min to 180 °C (2 min),

then ramped at 2 °C/min to 230 °C (25 min), and finally at 10 °C/min to 270 °C (15 min). The total run time was 75.5 min. The results were not corrected based on the recoveries of α -PDHCH internal standard, which was used as the recovery standard to check loses of volatile organochlorines like α-HCH, lindane and HCB during the sample concentration steps.

RESULTS AND DISCUSSION

Eleven PCB congeners, 28, 52, 49, 101, 149, 118, 153, 138, 180, 170, and 194, and nine pesticides, α-HCH, HCB, σ-HCH, β+γ-HCH, endrin, dieldrin, DDD, DDE, and DDT were investigated in the samples (Fig. 1). Percentage frequency of chlorinated pesticides and PCBs in the eighteen liver samples of wild grass cutter examined, are presented in Table 2. Eighty six percent of the samples contained detectable amount of hexachlorobenzene (HCB). Lindane and isomers were observed in 52 % of the samples. All the samples contained detectable levels of p,p'DDE, p,p'DDD p.p'DDT, and high chlorinated PCB congeners (138, 153, 180). Low chlorinated PCB congeners (28, 49, 52) were detected in 40 % of the samples. Low chlorinated PCB congeners (101 and 149), and high chlorinated congeners (118, 170, and 194) were not detected in any samples. The rodenticide, Endrin was not detected in any of the samples. Dieldrin was detected in 96 % of the samples.

While DDT, its metabolites and PCBs were detected, the concentrations were generally very low (< 12 µg/kg wet weight) indicating relatively less contaminated environments. The levels of organochlorine pesticides and PCBs studied are displayed in Table 3. Levels of pesticides and PCBs all fell below the WHO (1976, 1989) maximum permissible limits. PCB Congeners 153, 138, 180 were dominant in all the *Thyronomys swinderianus* liver samples, and the concentrations recorded were very low. Low PCB concentrations found are consistent with lack of information on the use of these compounds in the sampling area. The ΣPCB was 0.12 µg/kg, with range of 0.01-0.04 µg/kg. Low mean level of 0.04 µg/kg HCB was recorded. Lindane (γ-HCH) and isomers β-HCH, σ-HCH had mean levels, 0.06, 0.23, and 0.07 µg/kg respectively. Low level of DDT and metabolites were observed. Although, the levels of DDT and metabolites were low, they were dominant in all the samples investigated (Figure 2). This suggests relatively minor use of these chemicals for agriculture in nearby areas. However, the level of total DDTs was higher than total PCBs in all the samples suggesting more anthropogenic impact from agriculture compared to industry in the sampled areas. The mean values of ΣPCB and ΣDDT were 0.12 µg/kg and 6.49 µg/kg respectively. The results confirm p,p'DDD as the major degradation product in 90% of the samples studied (Fig. 2.) with highest mean level of 4.43 µg/kg . However in alkaline environments DDT can also undergo chemical degradation through dehydrochlorination to DDE. The application of σ-HCH and lindane as crop protection chemicals is generally allowed with few restrictions, whereas use of mixtures consisting of α, β, σ-HCH has been prohibited globally since 1977 (Unyimadu, 2001). Lindane (γ-HCH) generally has a short half-life in soil (Table 1). The detection of low 0.06 µg/kg lindane is as expected. The DDT and its metabolites show the persistence of these chemicals since they have been banned since 1972. The high frequency of chlorinated biphenyls congener 138, 153, 180, indicate that they are more persistent in the environment compared to low chlorinated biphenyls congener 28, 49, 52 which occurred in only 40 % of the samples. The results of studies on wild life from Germany are consistent with results from the present study. Studies by Bruggemann, *et. al.*, (1974) on different wild animals in Germany showed a wide occurrence of residues HCB, DDT and isomers in their liver compared to other tissues. Lower frequency was found for aldrin and dieldrin, however the rodenticide endrin was rarely found. Game animals generally have low levels of organochlorine contamination and this has been reported by Muller (1985a) and Rinkus *et. al.*, (1985). However Ewald (1976) recorded high levels of residues, which exceeded the permissible levels in Hare (*Lepus europaus Pall*) and Roedeer from Niedersachen, Germany. Holm, (1983) also reported high residue levels in Nord-Rheinwestfallen, Germany, compared to levels in Bayern and Hessen Germany. Results of residue analyses obtained from Roedeer and Hare from Schleswig-Holstein (Rinkus & Wolf, 1985, 1986, 1987a) showed average α-HCH of 750 µg/kg fresh weight. They were of the opinion that this high result was from the possibility of metabolism of γ-HCH to α-HCH during storage of samples. Low level of α-HCH was reported by Muller (1985a) in wild Boar from Saarland area, Germany. Bruggemann, (1974), Rinkus, (1985), Brunn *et. al.*, (1988) observed that high chlorinated biphenyls congener 138, 153, 180, were the main PCB residues in wildlife. No information has been published on the presence of organochlorines in game animals from Ore area of Ondo State. This report

Table 1. Half-life (t½ d) in soil of organochlorine pesticides investigated.

Pesticide	Half-life (t½ d) in days	Reference
HCB	600	Wallnofer, *et. al.*, (1989)
γ-HCH	110-345	Cliath & Spencer, (1971)
β-HCH	50-70	Guenziel, *et. al.*, (1971)
α-HCH	450-700	El Zorgani, (1976)
Dieldrin	140	Agnihotri, *et. al.*, (1977)
Endrin	140	Gowda & Sethunathan, (1977)
pp`DDE	320	Guenzi & Beard, (1976)
pp`DDD		Mitra & Raglu, (1986)
pp`DDT		Kaushik, (1991)

Table 2. Percentage frequency of Residues in liver samples of *Thyronomys swinderianus*

Residues	Frequency (%)
HCB	86
Lindane + Isomers	52
p,p'-DDE	100
p,p'-DDD	100
p,p'-DDT	100
Endrin	0.0
Dieldrin	96
Low chlorinated PCB (28,49, 52)	40
High chlorinated PCB (138, 153,180)	100

N = Number of samples, 20

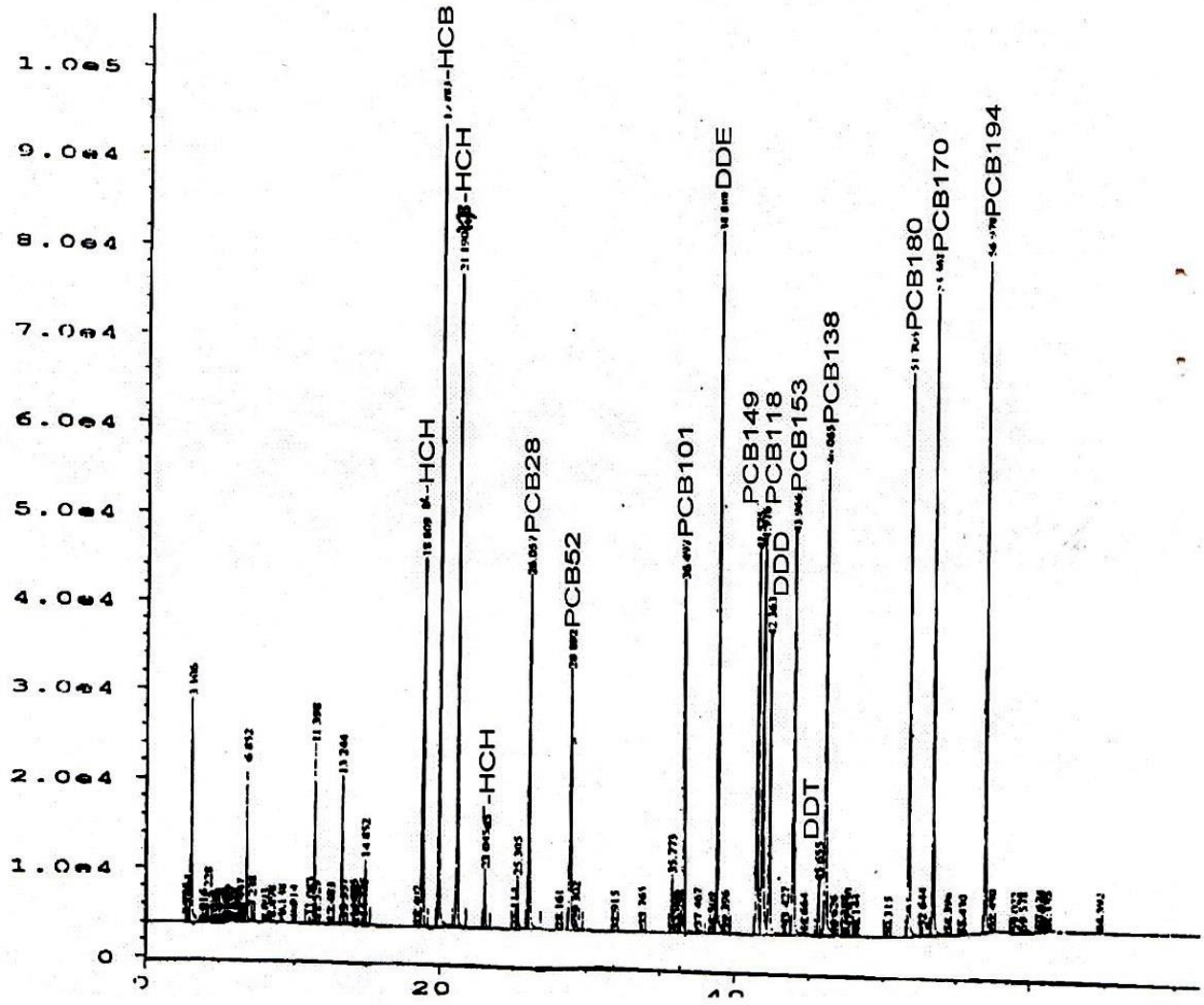

Fig. 1 Chromatogram of standards used for the quantification of organochlorine pesticides and polychlorophenols

serves as background data on the concentration of a range of organochlorine compounds in game animals from the area. The significance of organochlorine concentrations in biota is poorly understood. There are few data relating body burdens in game to either lethal or sub-lethal toxicity. Thus, it is not possible to access the implications of the data presented here for wild life populations, except that acute effects appear unlikely.

CONCLUSION

The residue levels in the twenty liver samples of grass cutter from Ore area analyzed were all below WHO maximum acceptable levels. DDT and metabolites were the main contaminants in the samples. p,p'DDD was the highest contaminant. Total DDT was higher than ∑PCB in all the samples signifying more

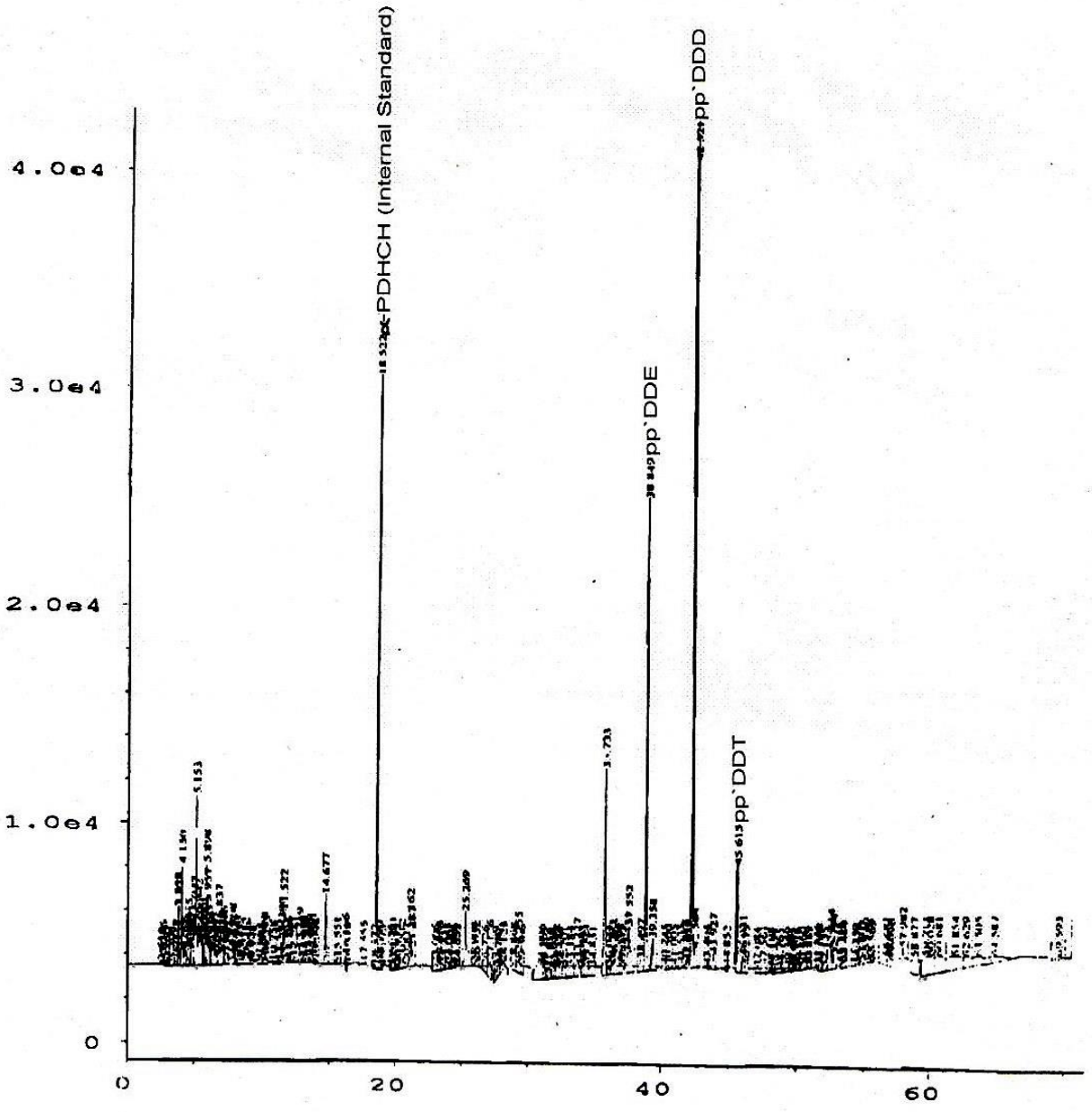

Fig. 2 The chromatogram of liver extracts (*Thyronomys swinderianus*) showing prominence of DDT and metabolites compared to other organochlorines

Table 3. Concentration of Residues in μg\kg wet weight of liver samples of Grass Cutter (*Thyronomys swinderianus*) with WHO permissible limits

Residues	Mean	Min/Max. value(μg/kg)	WHO (1976, 1989) μg\kg
HCB	0.04	0.03,0.06	200
γ-HCH	0.06	0.05,0.10	200
β-HCH	0.23	0.21, 0.26	100
α-HCH	0.07	0.05,0.09	200
Dieldrin	0.10	0.09,0.23	200
p,p'DDE	2.18	1.19,3.23	-
p,p'DDD	3.43	2.88,4.78	-
p.p'DDT	1.18	1.04,2.40	-
ΣDDT	**6.79**	**5.11,10.41**	**1000**
PCB 28	0.01	0.01,0.02	10
PCB 49	0.01	0.01,0.02	10
PCB 52	0.02	0.01,0.03	10
PCB 138	0.01	0.01,0.02	10
PCB 153	0.04	0.03,0.06	10
PCB 180	0.03	0.02,0.05	10
Σ PCB	**0.12**	**0.09,0.20**	**200**

anthropogenic effects from agriculture compared to the industry. The organochlorines are suspected to have been accumulated by the game through the food chain, because of intensive use of agro-chemicals in palm plantations nearby coupled with global atmospheric transport and deposition (Atlas & Giam, 1989). The residue analyses of Grass Cutter liver should be integrated in future environmental monitoring programs globally. In conclusion, the data presented here on the distribution, particularly of DDT and metabolites, provide a bases for assessing future data and targeting any further investigations.

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