Chlorinated Hydrocarbon Residues in Perianal Fat of Desert Bighorn Sheep

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Use of chlorinated hydrocarbon pesticides for more than 30 years and polychlorinated biphenyls (PCB) for more than 40 years has resulted in the accumulation of these compounds in various ecosystems. Their accumulation in different ecosystems both near and well removed from their source of application is enhanced principally by their persistence and subsequent magnification between trophic levels. Particularly noted for their residual properties are DDT and dieldrin as they can remain in their original state in the soil or be converted to various breakdown products. Photodecomposition of dieldrin results in products more toxic than the parent compound (SOTO and DIECHMAN 1967:307).

Although numerous investigators have inventoried chlorinated hydrocarbon pesticide residue levels in various big game species (CLAUSEN et al. 1974, SMITH et al. WALKER et al. 1965, BROWN and BROWN 1970), there is no information on the unintentional exposure and accumulation of pesticides nor the accumulation of chlorinated hydrocarbon residues in desert environments inhabited by desert bighorn (Ovis canadensis cremnobates and O. c. nelsoni).

Southern California desert bighorn occupy an altitudinal zone between 240-1200 m. Bighorn habitat is typified as being inaccessible and generally removed from the influences of man, especially those of an agricultural orientation. Despite their remoteness they are potentially exposed to pesticide residue in their food as a result of wind blown contamination from agricultural areas and from ground water contamination as a result of leaching and run-off from higher forested areas. Neither the direct nor cumulative effects upon bighorn from wide spread use of chlorinated hydrocarbon pesticides is well known.

The objectives of this study were to determine the extent to which o,p'DDT, p,p'DDT, p,p'DDE, p,p'DDD, PCB, aldrin, dieldrin, heptachlor, heptachlor epoxide and lindane are found in the perianal fat of two different races of desert bighorn sheep in Southern California deserts.

MATERIALS AND METHODS

Samples (3-10 gms) of subcutaneous perianal fat were collected from carcasses of 15 desert bighorn sheep. Additionally, biopsies (3-5 gms) of perianal fat were obtained from 6 free-ranging desert

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bighorn (Table 1) which were immobilized in conjunction with another study. Fat samples were enclosed in tight fitting new glass vials and stored at 13-20^{o}C until analyzed. Before analysis each fat sample (3 gms) was homogenized for 2 minutes with 40 mls nanograde hexane and anhydrous sodium sulfate in an ice jacketed flask. Tissue was transferred to a Buchner funnel with Whatman No. 1 filter paper; flasks were quickly rinsed with an additional 5 mls nanograde hexane; and cooled homogenate was rapidly filtered. The filter paper was then rinsed with 5 mls nanograde hexane. A 5 ml aliquot of filtrate was evaporated to dryness in a vacuum oven for gravimetric determination of total fat, and the remaining filtrate was cleaned up by using the technique of WOOD (1969).

Standards of heptachlor, heptachlor epoxide, lindane, aldrin, dieldrin, PCB (Aroclor 1254), o,p'DDT, p,p'DDT, p,p'DDE and p,p'DDD were added to mutton fat in known amounts to produce increasing levels from 0.1-5.0 ppm of each residue. One set of standards was extracted with each set of ten unknown samples. These extracted standards were corrected for background contamination in the mutton fat and then used as standards in the GLC and TLC analyses described below. Because the standards were added to mutton fat and experienced the extraction procedure, use of these standards automatically corrected for extraction efficiency.

All samples were separated into several groups by thin-layer chromatography (TLC) to reduce overlapping GLC chromatograms, principally those of PCB and DDT related compounds (REYNOLDS 1969). The TLC technique of BREIDENBACH et al. (1964) and the modifications of GREENWOOD et al. (1967) were used.

Residue concentrations were determined on a Hewlett Packard Model 7600A gas-liquid chromatograph equipped with a ^{63}Ni electron capture detector and an electronic integrator. A borosilicate glass column (2m x 4mm, i.d.) was placed with 10 percent DC-200 on 80 to 100 mesh gas chrom Q and used with a carrier gas mixture of 95 percent argon and 5 percent methane at 120 ml/min flow rate. The column, inlet and detector temperature were 200^{o}, 240^{o} and 250^{o}C respectively.

Standard curves were prepared from the integrator counts and known standards, and the unknown values were obtained from these curves. All residue concentrations were then calculated on a per-fat basis by dividing the whole sample concentration by the fraction of fat in the sample. Significant relationships were determined by use of non-parametric Mann-Whitney U statistics.

RESULTS

Results of desert bighorn perianal fat analysis are shown in Table 2. All samples analyzed had chlorinated hydrocarbon residues. Only one sample, from Los Angeles County (ocn-19), had heptachlor and heptachlor epoxide above the limits of detection (0.01 ppm). No samples had aldrin in detectable quantities (0.01 ppm).

Dieldrin and lindane were found in 17 (71%) and 22 (92%) of the 24 analyzed samples, respectively, but comprised an average of less

TABLE 1

Age, sex and county of desert bighorn biopsied and sampled for chlorinated hydrocarbon pesticide residue in Southern California.

Species	Sample No.	Age (yrs)	Sex	County
Penninsular Desert Bighorn (<u>Ovis canadensis cremnobates</u>)	occ 19*	3	E	Riverside
	occ 22	lamb	E	San Diego
	occ 26	4	E	Riverside
	occ 30	5	E	Riverside
	occ 34*	2	E	Riverside
	occ 37*	3	E	Riverside
	occ 39	lamb	R	Riverside
	occ 41*	6	R	Riverside
	occ 46*	11	R	Riverside
	occ 47	lamb	R	Riverside
	occ 50	3½	E	Riverside
	occ 51	lamb	R	Riverside
	occ 54*	4	R	Riverside
	occ 55	9	R	Riverside
	occ 59	5	R	Riverside
Nelson's Desert Bighorn (<u>Ovis canadensis nelsoni</u>)	ocn 19	lamb	R	Los Angeles
	ocn 22	6	R	San Bernadino
	ocn 36	6	R	Riverside
	ocn 39	4	E	San Bernadino
	ocn 51	3	E	San Bernadino
	ocn 57	3½	E	San Bernadino
	ocn 64	4	E	Inyo
	ocn 76	year.	R	Riverside

*Biopsy

TABLE 2

Residue levels of chlorinated hydrocarbon pesticides and PCB in perianal fat of desert bighorn sheep. Residues expressed in ppm and based on extracted fat.

Sample No.	Heptachlor	Heptachlor epoxide	Aldrin	Dieldrin	Lindane	DDT†	DDD*	DDE**	PCB	Total
occ 46	nd	nd	nd	0.05	0.03	0.01	0.04	0.08	0.41	0.62
occ 41	nd	nd	nd	0.01	0.02	0.01	0.03	0.03	0.39	0.49
occ 54	nd	nd	nd	nd	nd	0.02	0.03	0.05	0.21	0.31
occ 34	nd	nd	nd	nd	nd	nd	nd	0.03	0.22	0.25
occ 37	nd	nd	nd	0.03	0.01	0.01	0.04	0.02	0.13	0.24
occ 19	nd	nd	nd	0.05	0.04	0.02	0.01	0.04	0.25	0.41
occ 50	nd	nd	nd	0.04	0.04	0.03	0.01	0.06	0.27	0.45
occ 30	nd	nd	nd	0.03	0.02	0.03	0.02	0.07	0.65	0.85
occ 59	nd	nd	nd	0.06	0.03	0.01	0.02	0.03	0.19	0.31
occ 22	nd	nd	nd	0.03	0.03	nd	0.01	0.05	0.52	0.64
occ 26	nd	nd	nd	nd	0.01	nd	nd	0.02	nd	0.03
occ 31	nd	nd	nd	0.02	0.04	0.02	nd	0.05	0.33	0.46
occ 47	nd	nd	nd	0.03	0.04	0.02	nd	nd	0.16	0.25
occ 39	nd	nd	nd	nd	0.07	0.01	0.04	nd	0.13	0.25
occ 51	nd	nd	nd	0.04	0.03	0.01	nd	nd	0.42	0.50
occ 55	nd	nd	nd	0.02	0.01	0.02	0.03	nd	0.26	0.34
mean	—	—	—	0.03	0.03	0.01	0.02	0.03	0.28	0.40
(s)	—	—	—	(0.02)	(0.02)	(0.01)	(0.02)	(0.03)	(0.16)	(0.20)
ocn 64	nd	nd	nd	0.07	0.02	nd	0.04	0.07	0.82	1.02
ocn 76	nd	nd	nd	0.03	0.02	nd	nd	0.02	0.14	0.21
ocn 36	nd	nd	nd	nd	0.03	0.01	nd	0.06	0.19	.29
ocn 22	nd	nd	nd	0.04	0.07	0.03	nd	0.04	0.81	.99
ocn 51	nd	nd	nd	nd	0.03	0.01	nd	0.03	0.41	.48
ocn 39	nd	nd	nd	0.02	0.02	0.01	nd	0.05	0.33	.43
ocn 57	nd	nd	nd	nd	0.04	0.02	0.02	0.04	0.24	.36
ocn 19	0.09	0.06	nd	0.09	0.03	0.01	0.04	0.07	2.65	2.89
mean	—	—	—	0.03	0.03	0.01	0.01	0.05	0.70	0.83
(s)	—	—	—	(0.03)	(0.02)	(0.01)	(0.02)	(0.02)	(0.83)	(0.89)

nd = none detected; (s) = standard deviation; † = DDT residue values expressed as the sum of o,p'DDT and p,p'DDT; * = p,p'DDD; ** = p,p'DDE.

than 14 percent of the total residue load per animal. Levels of dieldrin and lindane were not significantly different between the two races of sheep ($p \leq 0.05$) averaging 0.03 ppm for both compounds.

DDT (reported as the sum of o,p'DDT plus p,p'DDT) or its decomposition products DDE (p,p'DDE) and DDD (p,p'DDD) were found in all analyzed fat samples. Levels of DDT, DDE and DDD were not significantly different between the two races of bighorn ($p \leq 0.05$). Average levels of DDT were less than either DDE or DDD. Residues of DDE contributed more than 56 percent of the residue attributed to DDT related compounds. The overall contribution of these compounds to the total residue load per animal averaged 17 percent. The residue load contributed by the DDT related compounds comprised a greater percent of the total load (20%) for the Penninsular bighorn when compared with Nelson's bighorn (13%), however, there was no significant difference in the absolute amount of these residues present ($p \leq 0.05$).

No significant relationships were found between rams and ewes for any of the residue levels determined (Table 3). Similarly, no age relationships were found for lambs and yearlings taken as a group, considered against adult animals (>2 years), except for DDE. The DDE levels (0.04 ppm) in adults averaged nearly four times the average residue level in lambs and yearlings (0.01 ppm); however, there was considerable variation in individual animals. The largest DDE residue recorded for a lamb was 0.05 ppm. This value was the sixth largest value recorded from the 24 samples analyzed.

Polychlorinated biphenyls were found in all samples analyzed and comprised an average of 69 percent of the total residue load per animal. The pennisular and Nelson's bighorn averaged PCB residue levels of 0.28 and 0.70 ppm, respectively. A Nelson's bighorn ram lamb, from Los Angeles Co. had the largest PCB residue level measured. Neither age, sex nor subspecies were significantly ($p \leq 0.05$) related to the measured PCB levels.

Total residue levels showed no significant relationship with sex or age group. Lambs and yearlings averaged 0.79 ppm total residue whereas adults averaged 0.46 ppm. When the average total residue was recalculated for lambs and yearlings without the unusually high residue values for sample ocn-19, total residues average 0.37 ppm. The significance of total residue levels to sex or age group was not importantly altered with the omission of sample ocn-19 from consideration.

DISCUSSION

Although chlorinated hydrocarbon residues are widespread in the ecosystem, 75 percent of the total land in the United States has never been sprayed with pesticides (USDA 1968). Data presented here indicate remoteness and inaccessibility do not preclude the inadvertent contamination of animal populations as a result of pest control operations.

Direct application of chlorinated hydrocarbon pesticides to desert bighorn habitat has probably never occurred; however, wind

TABLE 3

Comparison of mean chlorinated hydrocarbon residues per desert bighorn segregated on the basis of age and sex. Values in ppm and expressed on the basis of extracted fat. Standard deviation is in parentheses.

	Dieldrin	Lindane	DDT	DDD	DDE	PCB	Total
Lambs and Yearlings	0.04 (0.03)	0.04 (0.02)	0.01 (0.02)	0.02 (0.03)	0.01 (0.02)	0.67† (0.98)	0.79† (1.04)
Adults (>2 years)	0.02 (0.02)	0.03 (0.02)	0.01 (0.01)	0.02 (0.02)	0.04 (0.02)	0.34 (0.22)	0.46 (0.26)
Rams	0.03 (0.03)	0.03 (0.02)	0.01 (0.01)	0.02 (0.02)	0.03 (0.03)	0.50 (0.70)	0.62 (0.75)
Ewes	0.02 (0.02)	0.03 (0.01)	0.01 (0.01)	0.01 (0.02)	0.04 (0.02)	0.35 (0.23)	0.47 (0.27)

†Values calculated for PCB and total residue not considering ocn-19 are 0.27 (0.18) ppm and 0.37 (0.19), respectively.

and mediated vectors undoubtedly are, in part, responsible for the presence of these compounds in bighorn habitat. High levels of chlorinated hydrocarbon residues were found in 16 air samples from 18 California cities (WEST 1968). The observed high levels of chlorinated hydrocarbon residues in rain water, due to aggregation of residues on dust particles, is thought to be a major mode of residue transport in the ecosystem (COHEN and PINKERTON 1966).

The movement of residues from the soil to plants is rate limiting in the biological transport of residues in terrestrial organisms (MATSUMURA 1975). Although terrestrial plants usually do not accumulate chlorinated hydrocarbon pesticide residues above the levels found in soil, their contribution is significant because of their large biomass.

Acute pesticide toxemia in wildlife is generally accidental and occurs within a limited geographical area. Such localized exposure does not result in the same widespread ecological impact that chronic exposure and subsequent bioaccumulation of chlorinated hydrocarbon pesticide residues can cause.

It is well documented that residues of DDT and other chlorinated hydrocarbon pesticide residues accumulate and persist in adipose tissue (HAYES 1959, MATSUMURA 1975, PILLMORE and FINLEY 1963), but terrestrial mammals do not accumulate these residues to the extent of their avian counterparts (MATSUMURA 1975). Terrestrial herbivores generally accumulate the least amounts of chlorinated hydrocarbon residues, presumably due to their low level exposure within the trophic level.

Low levels of DDT and its related residues in desert bighorn sheep (0.01-0.13 ppm) are not unusual due to the widespread distribution of these compounds. Similar observations have been made by other investigators on various game species (BENSON and SMITH 1972, CLAUSEN et al. 1974, GREENWOOD et al. 1967, MOORE et al. 1968, TURNER 1965, WALKER et al. 1965).

A comparison of the proportions of DDT, DDD and DDE in the body fat allows for an assessment of the exposure of desert bighorn to these compounds (HUNNEGO and HARRISON 1971). The relatively high levels of DDE compared to DDT and DDD suggest that the bighorn's exposure was to either DDE or long term exposure to low levels of DDT, presumably the latter.

Aldrin is readily converted to dieldrin by plants and animals (BANN et al. 1956) and is presumably a factor in the absence of this compound (aldrin) from the samples analyzed. Dieldrin, however, is one of the most persistent chlorinated hydrocarbons known. Its frequency and low residual level in bighorn fat suggest a continual low level rate of intake (GANNON et al. 1959).

Comparatively high chlorinated hydrocarbon residue values were found in lambs. These residue levels indicate exposure was perhaps chronic, commencing with the lamb's first suckling or before. High residual levels of DDT and its derivatives have been shown to occur in milk (QUINBY et al. 1965). Similarly, lactation is a major pathway of dieldrin excretion (BRAUND et al. 1968, HARR et al. 1970, MURPHY and KORSCHGEN 1970). Transplacental movement of chlorinated hydrocarbon pesticides has been demonstrated in various animal groups (BRAUND et al. 1968, HATHWAY 1965, MURPHY and KORSCHGEN 1970). Placental transfer of chlorinated hydrocarbon residues and subsequent incorporation by fetal fat, in addition to lacteal augmentation, may have contributed to the high pesticide residue levels observed in the lambs.

Body fat in wild animals is a dynamic tissue which is rapidly mobilized as an energy source during illness, starvation or breeding activity. This can result in an increased concentration of chlorinated hydrocarbon residues in the remaining tissues (DALE et al. 1962). Otherwise diffuse low levels of residues could be potentially concentrated above benign levels to cause debility.

The United States Food and Drug Administration has set tolerance levels (maximum permissible amount) for chlorinated hydrocarbon pesticide residues in fat of livestock involved in interstate commerce; no such guidelines exist for game species. No levels of dieldrin or heptachlor are permitted in livestock fat. If the federal regulations were applied to harvested game species,* many would not meet minimal standard tolerance levels.

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*It should not be inferred that the Desert Bighorn sheep in California is a hunted game species. On the contrary, the bighorn in California enjoys a fully protected status. Unauthorized possession of bighorn remains is punishable under existing State laws.

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