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# Transplacental Movement of Organochlorine Pesticide Residues in Desert Bighorn Sheep

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The enormity of the earth has in part fostered the tradition of unconcern about the release of toxic wastes into the environment. Although some pollutants do disappear, many contaminants are persistent. Despite eventual dilution in the environment, biological systems are often capable of concentrating residues to sublethal or lethal concentrations.

Organochlorine pesticides and PCBs are considered among the most residual and are subject to concentration between trophic levels. Although concentrations do not normally achieve lethal levels, one of the most important parameters for assessing the sublethal effects of pesticides is the extent to which reproduction is affected. For large ungulate game species, this has rarely been investigated.

The bighorn is considered to be a species remote from the influences of man. Nevertheless, levels of organochlorine pesticide and PCB residues have been detected (TURNER 1977). High levels of residues in bighorn lambs suggest chronic exposure to pesticides commencing with a lamb's first suckling or before. High residual levels of various residues have been shown to occur in milk with lactation being a major pathway of excretion (QUINBY et al. 1965, BRAUND et al. 1968, HARR et al. 1970, MURPHY and KORSCHGEN 1970).

Transplacental movements of organochlorine residues has been demonstrated in several animal groups (PILMORE and FINLEY 1963, BRAUND et al. 1968, HATHWAY 1965, MURPHY and KORSCHGEN 1970), but it is not a widely accepted phenomenon. The placenta is a highly specialized group of membranes which demonstrate considerable specificity, the selectivity being related to the specific placental type.

The objectives of this study were to 1) determine distribution and concentrations of o,p'DDT, p,p'DDT, p,p'DDE, p,p'DDD, PCB, aldrin, lindane, dieldrin (HEOD), heptachlor and heptachlor epoxide in various desert bighorn (Ovis canadensis cremnobates) maternal and fetal tissues, 2) discuss the physiological and ecological consequence of such levels and 3) suggest a mechanism for placental lipid transfer.

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## MATERIALS AND METHODS

A 6½ year-old desert bighorn ewe and her term ram fetus were obtained for study. Both the ewe and fetus died as a result of dystocia due to a malpositioned fetus and a bacterial bronchopneumonia (Case Rec. No. 65859, County of Los Angeles, Department of Health Services).

Tissue samples (10-100 g) were collected fresh from the ewe and fetus and stored frozen at -20°C in tight fitting new glass vials until prepared and analyzed. Before analysis each tissue sample was homogenized for 2-5 min with 40 ml nanograde hexane and anhydrous sodium sulfate in an ice jacketed flask. Tissue was transferred to a Buchner funnel and Whatman No. 1 filter paper; flasks were quickly rinsed with an additional 5 ml chilled hexane, and cooled homogenate was rapidly filtered. The filter paper was then rinsed with 5 ml chilled 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 further purified by 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 carried through 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% DC-200 on 80 to 100 mesh Gas Chrom Q and used with a carrier gas mixture of 95% argon and 5% methane at 120 ml/min flow rate. The column inlet and detector temperature were 200°, 240° and 250°C, respectively.

Analysis on all tissues was done in triplicate. Values reported are the mean residue concentration levels for the three determinations.

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 fat-weight basis by dividing the whole sample concentration by the fraction of fat in the sample.

## RESULTS

Quantitative analysis for organochlorine residues are summarized in Table 1. All 14 maternal and 13 fetal tissues analyzed contained some residues. Aldrin, heptachlor and its epoxide were not found in quantities above experimental limits in any of the tissues examined. Absolute residue levels were greatest in the maternal tissues, except for 1) HEOD and lindane in fetal liver, 2) lindane in the fetal perianal fat and 3) HEOD and DDT in fetal omental fat. The greatest maternal residue load was measured in the gonad (0.55 ppm), whereas the greatest fetal residue load (0.40 ppm) was found in the cardiac and perianal fat (Fig. 1). Average total residue loads were 0.48 and 0.37 ppm for the ewe and fetus, respectively. Total fetal tissue residue loads averaged 76 percent (87-66%) of the corresponding maternal tissue.

HEOD and lindane were not ubiquitous in their distribution in either fetal or maternal tissues. With the exception of perianal fat and gonads, these compounds were not found in tissues of less than 10 percent fat content and at absolute levels not exceeding nor averaging less than 0.01 ppm. They contributed an average of 3 and 4 percent to the total maternal and fetal residue load, respectively (Table 1).

Similar to HEOD and lindane, DDT (reported as the sum of o,p'DDT plus p,p'DDT) and its alteration product DDD (p,p'DDD) were not universally distributed in the various tissues. DDT was found in greatest concentration (0.02 ppm) in maternal renal fat and placental tissue, but was not at levels above 0.01 ppm in any fetal tissues. DDD did not exceed 0.04 ppm in maternal and 0.03 ppm in fetal tissues. Generally, the greatest absolute amounts were confined to tissues of greater than 50 percent fat.

DDE (p,p'DDE) was found in all maternal and fetal tissues. The greatest absolute amount (0.04 ppm) was found in fetal perianal fat.

The overall contribution of the DDT related compounds to the total residue load averaged 14 percent (10-17%) for maternal tissues and 10 percent (3-20%) for fetal tissue. DDD and DDE comprised 84 percent and 87 percent of the maternal and fetal DDT compounds respectively.

Polychlorinated biphenyl (Aroclor 1254) was the greatest residue measured in fetal and maternal tissues, both in percent composition and absolute amount. PCB averaged 85 percent (79-90%) and 88 percent (78-97%) of the total residue loads for maternal and fetal tissues, respectively. The greatest fetal levels were found in the brain and the spleen (0.35 ppm). However, the greatest maternal levels were located in the gonad (0.44 ppm), and perianal and dorsal subcutantous fat (0.43 ppm). In all tissues the fetus contained less PCB than the maternal tissues.

TABLE 1

Residue levels of organochlorine pesticides and PCB in maternal and fetal tissues of a desert bighorn sheep. Residues are expressed in ppm and based on extracted fat. Fat concentrations are expressed as gms fat/100 gms tissue.

| Tissue | Fat | HEOD | Lindane | DDT+ | DDD* | DDE** | PCB | Total |
|---|---|---|---|---|---|---|---|---|
| EWE | | | | | | | | |
| Brain | 7.8 | 0.01 | nd | 0.01 | 0.03 | 0.03 | 0.38 | 0.46 |
| Heart | 9.9 | 0.01 | nd | 0.01 | 0.02 | 0.03 | 0.39 | 0.46 |
| Muscle[1] | 11.2 | 0.01 | 0.01 | 0.01 | 0.01 | 0.03 | 0.41 | 0.48 |
| Kidney | 2.5 | 0.01 | nd | 0.01 | 0.02 | 0.03 | 0.37 | 0.41 |
| Spleen | 3.1 | nd | nd | 0.01 | 0.03 | 0.03 | 0.41 | 0.48 |
| Liver | 4.0 | nd | nd | 0.01 | 0.04 | 0.03 | 0.40 | 0.48 |
| Gonad | 12.4 | 0.04 | 0.01 | 0.01 | 0.02 | 0.03 | 0.44 | 0.55 |
| Sm. Intestine | 6.3 | nd | 0.01 | 0.01 | 0.01 | 0.02 | 0.39 | 0.44 |
| Placenta[2] | 5.6 | 0.01 | 0.01 | 0.02 | 0.01 | 0.03 | 0.40 | 0.48 |
| Perianal Fat | 67.5 | 0.01 | nd | 0.01 | 0.04 | 0.03 | 0.43 | 0.52 |
| Cardiac Fat | 72.7 | 0.01 | 0.01 | 0.01 | 0.04 | 0.01 | 0.41 | 0.49 |
| Dorsal Subcu. Fat | 84.5 | nd | nd | nd | 0.04 | 0.03 | 0.43 | 0.50 |
| Omental Fat | 85.7 | nd | 0.01 | nd | 0.03 | 0.03 | 0.42 | 0.49 |
| Renal Fat | 79.6 | 0.01 | 0.01 | 0.02 | 0.04 | 0.03 | 0.42 | 0.53 |
| | | | | | | | Mean | 0.48 |
| | | | | | | | $\bar{s}$ | 0.04 |
| FETUS | | | | | | | | |
| Brain | 7.7 | 0.01 | nd | 0.01 | 0.02 | 0.01 | 0.35 | 0.40 |
| Heart | 7.5 | 0.01 | nd | 0.01 | 0.01 | 0.01 | 0.31 | 0.35 |
| Muscle[1] | 9.3 | 0.01 | 0.01 | nd | 0.01 | 0.01 | 0.30 | 0.34 |
| Kidney | 1.8 | nd | nd | nd | nd | 0.01 | 0.31 | 0.32 |
| Spleen | 2.8 | nd | nd | nd | nd | 0.01 | 0.35 | 0.36 |
| Liver | 9.4 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.34 | 0.40 |
| Gonad | 8.6 | nd | 0.02 | 0.01 | 0.02 | 0.02 | 0.31 | 0.38 |
| Sm. Intestine | 6.6 | nd | nd | nd | 0.01 | 0.01 | 0.31 | 0.33 |
| Perianal Fat | 65.4 | 0.01 | 0.01 | 0.01 | 0.03 | 0.04 | 0.31 | 0.41 |
| Cardiac Fat | 70.9 | 0.01 | 0.01 | 0.01 | 0.03 | 0.02 | 0.33 | 0.41 |
| Dorsal Subcu. Fat | 81.4 | nd | nd | nd | 0.01 | 0.01 | 0.31 | 0.33 |
| Omental Fat | 88.4 | 0.01 | 0.01 | 0.01 | 0.02 | 0.02 | 0.31 | 0.33 |
| Renal Fat | 70.4 | 0.02 | 0.01 | 0.01 | 0.01 | 0.02 | 0.33 | 0.40 |
| | | | | | | | Mean | 0.37 |
| | | | | | | | $\bar{s}$ | 0.03 |

nd = None Detected; + = DDT residue values expressed as the sum of o,p'DDT and p,p'DDT; *p,p'DDD; **p,p'DDE. [1]Sartorius muscle; [2]Placental cotyledons.

## DISCUSSION

Placental transfer of organochlorine residues has been investigated in a variety of domestic and laboratory animals experiencing chronic and/or acute dietary levels of various organochlorines (BRAUND et al. 1968, CURLEY et al. 1973, FINNEGAN et al. 1949, HATHWAY 1965, MURPHY and KORSCHGEN 1970). No studies, however, have documented transplacental movements of chlorinated hydrocarbon residues received "au naturel" in game species. Undoubtedly the difficulty of obtaining samples contributes to this void in our knowledge.

Data summarized in Table 1 suggest at least six organochlorine residues are capable of simultaneously passing the ovine placental barrier during the pre-partum period. The lack of discrete intimacy between the placenta (chorion) and the uterine epithelium of the chorioallantoic-syndesmochorial ruminant type placenta would, a priori, suggest that very little could pass the placental barrier in either direction by diffusion.

Placental circulation as well as the placental metabolism itself regulates the composition of materials experienced by the fetus. Placental metabolism is directly affected by maternal hormone levels and nutritional conditions (KORITNIK 1977). The placenta generally allows for the passage of electrolytes, minerals, amino acids and water against concentration gradients. Glucose, however, appears to diffuse across the placenta in response to a gradient. Proteins, with the exception of some antibodies, generally do not pass the placenta although the placenta has the capacity for substantial amino acid metabolism and interconversion. Amino acids appear to be actively and preferentially transferred (CURET 1970). Although the placenta is capable of lactate production and lipid synthesis (SZABO and GRIMALDI 1970) the placenta is impermeable to fats. The transfer of fatty acids and glycerol appears to be species dependent. As an example, the human placenta is seemingly impermeable to fats and the fetus utilizes primarily glucose for energy. Stored fetal fat is synthesized from glucose de novo by the fetal liver and is not absorbed directly from the maternal circulation (GUYTON 1976). In pregnant rabbits, however, fat seems to pass through the placental membranes from the maternal to fetal circulation (BELL et al. 1965). Similarly, fat soluble vitamins pass through the placenta. The constancy at which total fetal bighorn organochlorine residues approximate those of the maternal source suggest the possibility of an enzyme transfer system for lipids. This system presumably would involve phosphatase enzymes for specific lipids and residues moving passively with their associate lipids.

Placental transfer of HEOD, lindane, PCB and DDT and its related compounds occurred in the desert bighorn ewe resulting in the contamination of her fetus. The absence of local chemical industry and/or the application of pesticides within bighorn habitat suggests the presence of chronic levels of residues presumably imported at a regional level. Indeed, chronic levels of organochlorine residues within the region of southern California bighorn habitat have been demonstrated elsewhere (TURNER 1977). The

aggregation of residues on dust particles and their subsequent precipitation to earth by wind and rain is thought to be a significant transport mechanism for many xenobiotic compounds within the ecosystem (COHEN and PINKERTON 1966, YOUNG et al. 1976).

Bighorn migrations could serve to import residues from distant habitats and thereby contribute to the existing residue load, not only within the animal but within the habitat itself. However, the desert bighorn ewe's specificity of habitat preference, behavior and the physical restrictions imposed by the habitat itself preclude this from being a major contributory route.

None of the organochlorine residues detected, either individually or collectively, were of sufficient magnitude to cause acute debility within the ewe or fetus. Studies of domestic bovid species and several wild cervid genera would suggest much higher residue levels would be necessary to evoke an acute response (BRAUND et al. 1967, BRAUND et al. 1968, GREENWOOD et al. 1967, LABEN 1965, MATSUMURA 1975, MURPHY and KORSCHGEN 1970). Significantly higher levels of organochlorine residues apparently do not acutely alter population levels of many game populations nor adversely affect the consumption of wild game species by humans, although the latter point is difficult to assess due to the lack of federal guidelines on acceptable minimum tolerances for residues in game meat (GREENWOOD et al. 1967, PILLMORE and FINLEY 1963, TURNER 1977, WALKER et al. 1965). The total effect of chronic sublethal levels of most any environmental zenobiotic compound at the individual or population level still awaits clarification. However, it appears reasonable that chronic residue levels can be a negative influence on the energy flux within an animal community (ODUM 1972).

The accumulation of the DDT group of compounds is higher than the other pesticide residues found (Table 1), although the absolute levels were not great. DDT and DDD were the major contributing factors to the level of contamination by this group of pollutants. The low levels of DDT and related residues perhaps reflect background levels of contamination which are not necessarily unusual considering the once widespread use of DDT and its persistence in the biosphere and associated biomass. Bighorn sheep do not accumulate high levels of pesticides due to their low level of exposure within the trophic structure as herbivores. Residue ratios of the various DDT compounds suggest that the maternal exposure was to low levels of DDT over a long term (HUNNEGO and HARRISON 1971). The fetus' residues reflect the maternal exposure.

Polychlorinated biphenyl constituted the major source of residues found (Table 1). Studies of aquatic and terrestrial ecosystems indicate levels of organochlorine residues such as DDT, aldrin, lindane, etc. are declining as their use becomes more guarded (FRANK 1971). However, residues of PCB compounds are becoming more abundant in biological systems (REYNOLDS 1969, RISENBROUGH et al. 1968). Their activity appears to impair reproductive performance, although this is not their exclusive mode of action in biological systems. PCBs have been shown to induce the hepatic microsomal enzyme system in rats (STREET et al. 1969, LITTERST et al. 1972, CHEN and DUBOIS 1973, BRUCKNER et al. 1974a,

1974b, SCHMOLDT et al. 1974). Similar observations have been made in the rabbit (VILLENEUVE et al. 1971). High maternal and fetal levels of PCB residues have been blamed for the premature births of sea lions (DELONG et al. 1973).

PLATONOW et al. (1972) found sublethal levels of PCB (Aroclor 1254) reduced urinary levels of dehydroepiandrosterone and estrogen in the boar. It is presumed that PCBs affect enzyme systems which metabolize sex hormones. The activity of hydroxylating enzymes which metabolize estradiol is induced by PCB (RISENBROUGH et al. 1968). This has the potential of causing reproductive failure.

Chronic levels of PCB fed to corckrels resulted in anti-androgenic effects on the development of the testicles. Similarly, the post-microchondrial hepatic fraction (microsomes + cytosol) from PCB-treated cockrels and pullets metabolized testosterone, estradaion-17β and 4-androstene-3, 17-dion at an approximate three-fold increase over untreated birds (NOWICKI and NORMAN 1972).

The structural similarity of PCB and DDT suggest that they may have similar pathways of toxicity. The site of o,p'DDD inhibition of ACTH-induced steroidogenesis in the adrenal cortex appears to be at the ACTH mediated intramitochondrial conversion of cholesterol to pregnenolone (HART and STRAW 1971). Pregnenolone is a precursor of dehydroepiandrosterone in steroidogenesis. Circumstantially, it would appear to be at this level that PCB would have a major effect on reproduction.

The susceptibility to the toxic effects of PCB varies considerably across the vertebrate taxa for which there are comparable data. Mink experienced poor reproductive success on PCB levels approximately those found in desert bighorn sheep (PLATONOW and KARSTAD 1973, TURNER 1977). The toxic effects are less pronounced, however, in the domestic cow, pig and chicken (PLATONOW et al. 1973, PLATONOW et al. 1973, SASCHENBRECKER et al. 1971). This susceptibility appears to be related to the extent to which an animal metabolizes the PCB. The most toxic effects are seen in those species which extensively metabolize PCB. It would seem logical to rank the bighorn's metabolic capabilities with those of the cow. However, further studies would be required to substantiate this.

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