

# The importance of ecotoxicological research in management of the snow leopard: lessons learned from the Florida panther

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Conservation and management of endangered species generally focuses on issues such as habitat protection, genetic diversity, population dynamics, and interspecific competition. Research therefore is primarily devoted to the ecological and biological characteristics of the species, such as habitat characterization, habitat use, food habits, social behavior and breeding programs. Comparatively little attention is devoted to the abiotic factors that may be affecting the species of concern. The presence and impact of xenobiotic compounds in protected areas and the potential impact of these compounds on endangered species in protected areas may be overlooked. Anthropogenic chemicals that are known to disrupt biological systems are found throughout the world and can have very damaging consequences on exposed individuals or populations. Populations of endangered species are particularly at risk following exposure to such compounds. Therefore, management of endangered species should include an evaluation of potential chemical stressors on the viability and integrity of the species of concern. The importance of this aspect has been demonstrated to be an unanticipated critical factor in the management of the Florida panther (*Felis concolor coryi*) in protected areas in south Florida.

Recent developments in global assessment of the distribution and transportation of persistent chemicals in the environment have revealed important insights on long range transportation processes of xenobiotics from industrialized and urban areas to relative pristine areas. Results of these studies reveal that adsorption of persistent chemicals in lichens, such as DDT and HCH, in the Himalayan region are among the highest in the world. The concentration of persistent agrochemicals is proportional to the concentration in the ambient air. Since production and use of these persistent chemicals is still increasing in certain areas in the world, especially on the Indian subcontinent, accumulation and therefore an increase in the residue levels in remote areas is to be expected. Transport phenomena in the atmosphere on and around the Tibetan plateau are highly complex. Along with other long range transport phenomena, such as dry deposition, and possible use of persistent agrochemicals in agricultural areas in the Himalayas in the present and past, residue levels of these compounds may be ecologically significant. Establishment of the ecological impact of these compounds in protected areas on the Tibetan plateau may therefore be of crucial importance with respect to management practices for snow leopard.

At this point we propose a limited research program to establish the presence of persistent compounds in protected areas, as well as the present exposure levels of the snow leopard and its prey base.

## OCCURRENCE AND SOURCES OF PERSISTENT CHEMICALS IN SNOW LEOPARD HABITAT

The snow leopard (*Uncia uncia*) occurs in the remote, sparsely populated areas of the central Asian highlands (Green 1988). Two separated areas are to be distinguished. The southern area consists of the south-central provinces of the former USSR (Kazakhstan, Kirghizia, Tadzhikistan and Uzbekistan), Afghanistan, Pakistan, northern India, Nepal, Bhutan and the southeastern provinces of China (Qinghai, Gansu, Sichuan, and Xizang Autonomous Region). The northern area of occurrence consists of Mongolia and south Siberia. Efforts to protect the snow leopard have resulted in the establishment of a number of wildlife preserves, totalling approximately 15,000,000 ha. (Green 1988). The majority of the wildlife preserves are located at the south eastern boundary of the area of occurrence of the snow leopard, which corresponds to the Himalaya, Hindu Kush and Kopet Dag mountain ranges (Green 1988).

Global distribution of persistent xenobiotics, such as DDT, is so extensive that it is claimed that a qualified pesticide residue analyzer with the proper equipment could find measurable amounts of DDT in any non-fossil sample (Gunther 1966). Research assessing the distribution and enviro-dynamics of persistent xenobiotics in remote areas has received increasing attention during the last decade. Addison et al. (1984) report a decrease in Sigma-DDT and a stationary trend for PCBs in blubber of the predatory gray seal (*Halichoerus grypus*) and harp seal (*Pagophilus groenlandicus*) in the period between 1971 and 1982 in Nova Scotia, Canada. Norstrom et al. (1988) found an increase of Sigma-Chlordane (4x), other organochlorine compounds such as HCH, dieldrin and PCBs (2x) and a stationary trend in Sigma-DDT in adipose tissue of polar bears (*Ursus maritimus*) collected from 12 zones in the Canadian arctic and subarctic marine ecosystem over the period between 1969 and 1984. In terrestrial ecosystems, plant biomass is believed to play a significant role in circulation and bioaccumulation phenomena of persistent chemicals, and air to leaf transfer of gaseous organics can be considered a key process, particularly for less

soluble compounds (Buckley 1982, Bacci and Gaggi 1985, Erickson et al. 1989, Bacci et al. 1990, Riederer 1990, Trapp et al. 1990). A global survey of concentrations of Sigma-DDT, HCB and alpha- and gamma-HCH in lichens, mosses and mango-leaves has been conducted by Calamari et al. (1991). Samples were collected in trajectories of approximately 100 km in 36 locations in America, Africa, Europe, Asia and Antarctica. Three locations that coincide with areas in which snow leopards occur, all in Nepal, were sampled: Nepal-lowlands (NL), Nepal-mountains (NM) and mount Everest (ME). The results of this study indicate that the southern Himalaya area ranks among the most contaminated in the world with respect to HCH components, and is also in the upper rankings for Sigma-DDT. The highest concentrations of HCHs and DDT were found around New Delhi, India. Rankings for individual compounds in Nepal were: alpha-HCH (NL: 5, NM: 3, ME: 6), gamma-HCH (NL: 5, NM: 2, ME: 7), p,p'-DDT (NL: 7; NM: 10; ME: 17). Absolute concentrations ranged from 9.5-35.74 ng/g for alpha-HCH, 1.15-4.47 ng/kg for gamma-HCH and 2.10-13.70 ng/g for p,p'-DDT in the lichens from the three locations in Nepal. These results are rather alarming since this study included those areas in the world in which the use of these insecticides is known to be heavy (central and South America, west Africa).

Thomas (1986) mentions that uptake of the organochlorine compound benzo hexachloride (BHC) in plants results from the uptake of precipitation water rather than direct uptake of gaseous traces. Concentrations of polycyclic aromatic hydrocarbons are related to particle concentrations in the air. Numerous studies have been conducted to determine the sources of contaminants in remote areas, especially with respect to the arctic (Rahn and McCraffey 1980, Heintzenberg et al. 1981, Pacyna et al. 1985, Pacyna and Oehme 1988). Pacyna and Oehme (1988) argue that the presence of chlorinated hydrocarbons in the Norwegian arctic originates from the Ural and the Kola Peninsula. Welch et al. (1991) report the presence of brown snow near the Chesterfield inlet in the Canadian arctic and analysis of this material reveals the presence of a number of persistent chlorinated compounds and polycyclic aromatic hydrocarbons. Air mass trajectories, clay mineral composition, soot particles and visible organic remains point to Asian sources, probably China.

The origin for the relative high concentrations of Sigma-HCH and Sigma-DDT in the southern Himalaya is thus far unknown. Climate patterns in the Himalaya area are extremely complicated in terms of air circulation, and a seasonal variation of circulation patterns, changing from eastern direction to western direction is reported to take place twice a year (Gao You-xi and Li Ci 1981, Reiter 1981, Ye Du-zheng and Gao You-xi 1981). Frictional effects of the Tibetan plateau in relation to particle deposition may be important and result in a complex deposition pattern on and around the Tibetan plateau.

A possible source for organochlorine insecticides is India. HCH and DDT are still widely used in India and the demand is increasing. Presently, annual consumption amounts to about 31.6 million kg for HCH and 3.6 million kg for DDT (FAO 1988, ICS 1986). The use of cyclodienes such as aldrin is moderate, but increasing (FAO 1988). The tropical climate in this area enhances rapid transportation through water and air. Takeoka et al. (1991) developed a flux model for the Vellar estuary in south India and predict that 99.9% of the applied amount of HCH applied is removed and transported through the air. Studies by Saxena et al. (1987), Agarwal et al. (1987) and Kaushik et al. (1991) support the importance of atmospheric transportation for the environmental distribution of HCH's and DDT. High ubiquitous concentrations are presently becoming a serious hazard for human health in India. High concentrations of HCHs and DDT have been reported in edible oils and seed oils (Dikshith et al. 1989) and human milk (Zaidi 1989).

Another potential source for the presence of xenobiotics, such as HCH and DDT in the southern Himalaya is China. Welch et al. (1991) report China as a possible source for the brown snow phenomena. Wang (1981) reports the production and use of HCH over a long period. Han (1989) reports serious environmental problems as a result of accumulation of organochlorine residues in the agricultural environment, food and human bodies, especially in areas where crops such as cotton are grown. Guo (1988) estimates that the amount of pesticide-polluted arable land accounts for 13 million ha. and industry polluted land accounts for 6 million ha. In 1983, the Chinese government decided to ban the production of organochlorine pesticides, mainly DDT and 666 (Xu and Peel 1991). Agrochemical production shifted more toward organophosphates (72.8% of total pesticide output in 1984) and pyrethroids. Despite the ban on the production of organochlorine pesticides in 1983, China remains a potential source for the long range transportation of organochlorine pesticide residues. DDT is freely available at pesticides retailers in China (Marden, pers. obs.).

Other potential sources may be Nepal and the former Soviet Union. The fact that HCH concentrations are higher in the Nepali mountains than in the Nepali lowlands suggest a more remote

source for these insecticides. The highest concentrations of DDT are found in the Nepali lowlands and may be attributed to local sources. Consumption of HCH in the former Soviet Union is huge (Komarov 1980) but specific data on pesticide use in the former Soviet Union are unavailable. Recent information regarding the environmental condition of the former USSR indicates that until recently, DDT was commonly used in agricultural practice.

## **ECOLOGICAL SIGNIFICANCE OF PERSISTENT CHEMICALS FOR THE MANAGEMENT OF ENDANGERED SPECIES**

The importance of environmental contaminants for the management of endangered species can be demonstrated by the example of the Florida panther. The presence of mercury in protected areas in southern Florida has become a pivotal aspect in the management of this endangered species. The presence of mercury in the southern Florida ecosystem was totally unexpected and its importance has been overlooked until recently (Roelke et al. 1991). A report on the status of mercury contamination in the U.S. (EPA 1975) mentions Florida as one of the two states in the US with the lowest annual man-made mercury emission in water and air. Studies later demonstrated the presence of mercury in fish and bird species in the Everglades National Park and the Loxahatchee National Wildlife Reserve (Roelke et al. 1992). The sources of mercury contamination in Florida are not clearly established, although atmospheric deposition as a result of waste incineration and burning and processing of sugar cane, and oxidation of natural peat soils are mentioned as primary sources (Roelke et al. 1991). Global atmospheric concentrations of mercury have substantially increased over time (Slemr and Langer 1992), and may have contributed to the presence of mercury in southern Florida.

In the summer of 1989 a 3-4 year old female adult Florida panther was found dead in the Everglades National Park. Gross and histopathological examination were unremarkable. Liver tissue was analyzed for pesticides, PCBs and heavy metals. The liver contained 110 mg/kg mercury. Death due to mercury toxicosis in domestic cats has been reported for liver concentrations ranging from 37-145 mg/kg (Harada and Smith 1974). Mercury analysis was then performed on blood and hair samples collected from free-ranging Florida panthers between 1978 and 1991, panthers found dead, white-tailed deer (*Odocoileus virginianus*), raccoons (*Procyon lotor*), bobcats (*Felis rufus*), otters (*Lutra canadensis*) and alligators (*Alligator mississippiensis*). The results indicate that mercury toxicosis may have contributed to the death of three Florida panthers. Females with elevated mercury had poorer reproductive success than those with low mercury levels.

Mercury contamination in Florida panthers is related to the food chain. Panthers that mainly feed on white-tailed deer and feral hog showed the lowest concentrations of mercury. Although mercury concentrations in feral hogs have not been established, mercury levels in white-tailed deer in southern Florida are low. Panthers with elevated levels of mercury occur where they consume non-ungulate prey such as raccoons, which are thought to be the primary source of mercury. Adapted management strategies, aimed to enhance deer density, have contributed in a significant drop in mercury levels in Florida panthers living in the Fakahatchee Strand State Preserve. Chronic exposure to mercury, resulting in direct mortality, lowered reproductive success and other sublethal effects is a significant factor for lower than expected population densities of Florida panthers in large portions of their range and is likely contributing to the extinction of this endangered mammal (Roelke et al. 1992).

## **ENVIRONMENTAL EFFECTS OF ORGANOCHLORINE CONTAMINANTS ON TERRESTRIAL ECOSYSTEMS**

The environmental effects of organochlorine contaminants, such as DDT, aldrins and PCBs have been extensively studied. A survey of the findings of these studies falls well beyond the scope of this paper. A detailed review of the studies conducted can be found in Edwards (1973) and USEPA (1975).

The enviro-dynamics of organochlorine residues has been reasonably well established for small mammals in terrestrial ecosystems. Herbivorous and omnivorous species, such as *Peromyscus* spp., *Microtus* spp., *Lepus* spp., *Sylvilagus* spp. and *Citellus* spp. do not bioaccumulate pesticide residues, even in areas of heavy pesticide use (USDA 1969). Carnivores species, such as moles, *Blarina* spp., *Microsorex* spp. and *Sorex* spp. accumulate high residue levels of organochlorine pesticides (Dimond and Sherburne

1969, USDA 1969, Bailey et al. 1974). Several studies demonstrate that larger carnivorous mammals, such as mink (*Mustela vison*), polecats (*Mustela putorius*) and fox (*Vulpes vulpes*) can bioaccumulate high levels of organochlorine pesticides and PCBs (Blackmore 1963, Sherburne and Dimond 1969, Mason and Weber 1990).

A number of studies report residue values for organochlorine pesticides in big game animals. The results indicate a large variance in presence of organochlorine pesticides in adipose tissue (Edwards 1973). Pillmore and Finley (1963) conducted studies in several DDT-treated areas in the U.S. (Montana, Colorado and New Mexico) where they measured organochlorine pesticides, showing concentrations of DDT up to 43 mg/kg in mule deer (*Odocoileus hemionus*) and 29 mg/kg in elk (*Cervus canadensis*). A similar study has been conducted by Benson and Smith (1972) where DDT was sprayed at 1 lb/acre on a 525,000 acre area in Idaho. Adipose tissue from deer analyzed three months and five years post-application showed average DDT levels of 19.4 mg/kg and 0.2 mg/kg, respectively. Extensive studies in which the presence of organochlorine pesticides are determined in feline species in the wild have not been conducted.

DDT causes hepatomas, lung carcinomas and lymphomas in mice (IARC 1974). An increased incidence of hepatic tumors has been observed with doses of DDT as low as 2 ppm (USEPA 1975). DDE administered in high concentrations causes hepatocellular tumors late in life in Syrian hamsters, and also increased the incidence of adrenocortical adenomas (Rossi et al. 1983). DDT and metabolites are demonstrated to be and are used as tumor-promoters in rats (Hilpert et al. 1983, Kitagawa et al. 1984). DDT causes the DDT-syndrome in rats, consisting of tremor, myoclonus, running seizures, hypothermia, episodic boxing and excessive grooming (Truong et al. 1988). Sublethal effects of DDT, such as reduced weight gain, feed consumption, weight of lung, liver and spleen, and abnormal values of some biochemical parameters were observed in rabbits dosed 10 mg/kg and sacrificed on day 21 after exposure (Shung et al. 1989). One DDT metabolite (3-methylsulfonyl-DDE) causes adrenocortical toxicity in mice in ecologically relevant doses, as low as 3 mg/kg (Lund et al. 1988, Jonsson et al. 1991). Structural changes in the adrenal glands as a result of exposure to low concentrations of DDT (0.2 mg/kg/day during 120 days) have also been observed in rats (Chowdhury et al. 1990). Both o,p'-DDT and p,p'-DDT have been found to be estrogenic compounds (Bustos et al. 1988).

The locus of primary toxic action of DDT is believed to be sensory and motor nerve fibers and the motor cortex. DDT causes repetitive stimulation of the peripheral sensory nerves is magnified into the central nervous system (CNS), causing generalized tremoring throughout the body. There are at least four possible mechanisms causing this response (Matsumara 1985).

Dikshith et al. (1991) derived a No Observed Effect Level (NOEL) for technical HCH of 0.5 mg/kg/day for rats in a 360 day chronic experiment. Animals exposed to higher concentrations showed signs of intoxication and increased mortality was observed in the four highest exposure levels (5, 25, 250 and 500 mg/kg/day). Pathomorphological changes were observed in the two highest exposure levels. Exposure to HCH produces signs of poisoning similar to those of DDT, e.g., tremors, ataxia and convulsions. The gamma- and alpha-isomers are convulsant poisons; the beta- and delta-isomers are CNS-depressants (Amdour et al. 1991). Lifetime exposure studies in mice have revealed that technical HCH and some isomers caused an increase in hepatocellular tumors (IARC 1974). Gamma-HCH (lindane) suppresses the adrenocortical function in female mice (Lahiri and Sircar 1991). Gamma-HCH is believed to be an antiestrogenic compound in studies with female rats (Chadwick et al. 1988). HCH causes reproductive toxicity in male rats (Pius et al. 1990). All HCH compounds are inhibitors of brain-specific t-butylbicyclophosphorothionate (TBPS), indicating an action at the gamma-aminobutyric acid (GABA)-regulated chloride channel. This mechanism of toxicity is also observed for chlorinated cyclodiene pesticides. Mammalian toxicity is closely related to the potency of inhibition of TBPS-binding (Lawrence and Casida 1984).

Although this outline on the toxicological effects of DDT and HCH is based on only a minute fraction of the literature available on this topic, it demonstrates some of the potential effects that can occur in species chronically exposed to relative low concentrations of these compounds. Snow leopards are opportunistic feeders on a variety of species, including domestic animals, birds, small and large mammals, vegetation and carrion (Mallon 1984, 1988). The relative high concentrations of HCH and DDT in the vegetation of the mountains of Nepal, the expectation that these concentrations will most likely increase due to continued use of large amounts of these pesticides in especially India, and the potential of bioaccumulation in the food chain leave the snow leopard, being a top-predator, vulnerable for adverse toxic effects of bioconcentrated residues of HCH and DDT and possibly other persistent organochlorine compounds that have not been identified yet. These considerations demonstrate the need for establishing

background levels of organochlorine compounds in the food chain in wildlife reserves in the Himalaya area where the snow leopard occurs. No information is presently available regarding the presence of organochlorine contaminants in other areas where snow leopards occur, thus indicating the importance of conducting a preliminary assessment of the presence and concentration of contaminants in these areas as well.

## **RECOMMENDED ACTIONS**

An assessment of ecologically relevant organochlorine compound residues can be performed through two different approaches. An initial screening can be performed in order to determine the presence in the food chain of protected areas. If residue levels are sufficiently high to cause problems in higher trophic levels, a more detailed and thorough approach is recommended.

### **Initial screening for organochlorine residues**

Initial screening for organochlorine residues can be performed in an opportunistic manner by collecting tissues, preferably adipose or liver tissue, from animals found dead or killed in the protected areas. Depending on the local situation, the major prey items of the snow leopard should be included in such a survey. If snow leopards are immobilized for the purpose of examination or radio-tagging, blood samples, hair samples, and possibly a biopsy of adipose tissue can be collected. If none of these options are practical or available, residue analysis can be performed on liver tissue samples from domestic grazing animals that have been harvested for human consumption.

Residue analysis should be performed at a qualified laboratory on a broad spectrum of organochlorine compounds. Besides the compounds discussed in the previous sections, PCBs and, more recently, toxaphene compounds are also considered to be global contaminants. These compounds, which are complex organochlorine mixtures, interfere with the organochlorine pesticide analysis. Therefore, sample extracts need to be fractionated to provide reliable identification and quantification of residues.

At this stage, enzyme bioassays which indicate exposure to organochlorine compounds, such as ethoxy-resorufin-O-dealkylase bioassay (ERODE), the pentoxy-resorufin-O-dealkylase bioassay (PROD) and other cytochrome P450 bioassays, are not recommended. Although these bioassays are proven indicators of environmental exposure to DDT, HCHs and PCBs (Lubet et al. 1990), there are important practical limitations. Such bioassays require the capacity to prepare microsomal fractions from liver samples and store them at -80°C, or at the very least freeze liver samples immediately after collection. An additional requirement for accurate interpretation of enzyme bioassays is reliable control group of similar animals. Blood samples can be utilized to perform background enzyme levels and blood parameters, such as hematocrit and lymphocytes.

### **Ecosystem-level assessment of the adverse effects of organochlorine residues**

If initial screening reveals the presence of contaminant levels that may cause adverse effects in the functioning of ecosystems, a more detailed study would be required. Such a study needs to be carefully designed and implemented, and the results need to answer fundamental and pragmatic questions simultaneously. Mechanisms of transport, bioaccumulation and biomagnification of contaminants need to be evaluated, as well as practical aspects of adaptive management to minimize exposure related risks for endangered species in the protected areas. Management strategies will most likely not be focused on the snow leopard alone, but should also include other endangered species in these areas. Birds are generally more sensitive to the effects of organochlorine pesticides and have greater capacity for bioaccumulation (Walker 1983).

The recommended approach is bottom-up, starting with the environmental characterization of sediments and primary producers. Analysis of lake sediments allows determination of temporal deposition patterns of contaminants, when applied in combination with techniques such as Pb<sup>210</sup> radio-dating. After sufficiently establishing contaminant patterns in the lower trophic levels, contaminant levels in herbivores and ultimately, carnivores can be determined.

A thorough assessment of the environmental condition of these ecosystems can be very costly. A substantial commitment of time, labor and expertise is required to rigorously characterize the enviro-

dynamics and distribution of contaminants in the ecosystem. The example of the Florida panther has taught us that detailed information regarding both biotic and abiotic components are necessary to fully assess the vitality of ecosystems, and is of crucial importance for the management and survival of endangered species.

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