Bull. Environ. Contam. Toxicol. (2002) 68:377–382

DOI: 10.1007/s00128-001-0264-y

Bulletin of Environmental Contamination and Toxicology

Levels of *p,p′*-DDE in Liver of Predatory Birds from Calabria, Italy

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Received: 20 March 2001/Accepted: 30 September 2001

Predatory birds are often used as bioindicators of organochlorine compounds (OCs) environmental contamination. Notwithstanding favorable trends due to the restrictive legislation in developed countries, OCs are still present and must be kept under control. In addition, illegal and improper use of OCs can not be totally eliminated. In some developing countries many OCs are currently legally registered since they prove to have many advantageous uses, e.g., against the spreading of malaria, and as a result, are considered indispensable.

OCs also present cause for concern due to their environmental persistence and widespread global transport. There is evidence of the bioconcentration of some OCs in the higher trophic levels of terrestrial and aquatic food chains, especially birds. At relatively low concentrations, OCs can exert subtle toxicological effects on enzyme activity, hormonal balance (so called 'endocrin disruptors') and reproduction.

In the present study, the levels of OCs in the liver of predatory birds were evaluated. Liver samples were collected from 80 birds (9 different species) found dead or dying in the territory of the Calabria region of southern Italy from 1993 to 1997. Collected data were compared to data already available regarding the OCs contamination in predatory birds from Italy and other European countries.

MATERIALS AND METHODS

Raptors found dead or dying, usually due to injury (gun shots, traumas), during the period 1993-97, were necropsied at the local faunistic centers, and liver samples were collected, frozen, and quickly sent to the lab

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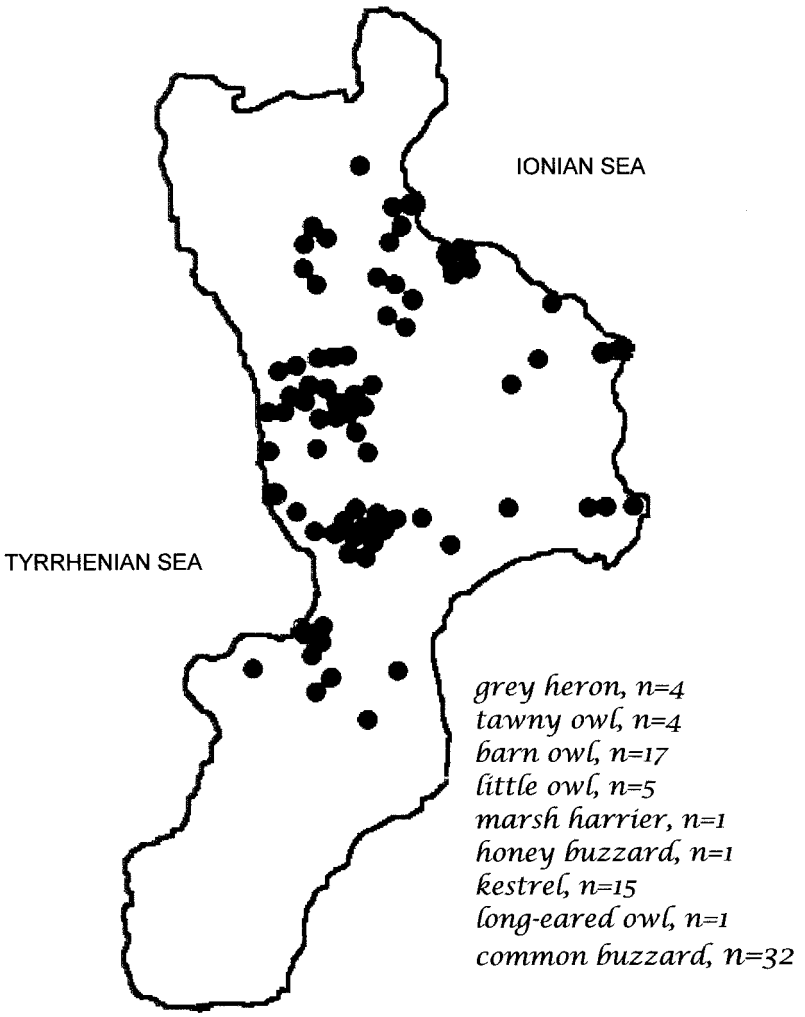

Figure 1. Calabria region: black spots highlight the sites where the birds were collected; n = number of samples.

where they were stored at –80°C pending analysis. The samples were taken from the following species: grey heron (*Ardea cinerea*), tawny owl (*Strix aluco*), barn owl (*Tyto alba*), little owl (*Athene noctua*), marsh harrier (*Circus aeruginosus*), honey buzzard (*Pernis apivorus*), kestrel (*Falco tinnunculus*), long-eared owl (*Asio otus*) and common buzzard (*Buteo buteo*). See Figure 1 for details.

Sample extraction was performed using with slight modifications the method recommended by A.O.A.C. (1996) for OCs in fat-containing foods. Extracts (1µl) were injected into a gas chromatograph (Carlo Erba 5160 Mega Series) equipped with a ^{63}Ni electron-capture detector. The

instrumental parameters and operational conditions were as follows: a CP-SIL-19-CB fused silica capillary column (25m x 0.32mm i.d., film 0.20 μm) and a temperature program from 60°C to 180°C at 10°C min^{-1}, followed by a run from 180 to 270°C at 4°C min^{-1}. The carrier gas was hydrogen at 1 ml min^{-1} and the make-up gas was nitrogen. Concentrations were calculated using fenchlorfos as internal standard. The percent recovery of *p,p'*-DDE was 82±4%.

In order to obtain a normal distribution, a log transformation of *p,p'*-DDE concentrations was applied. Differences in contamination levels among species were tested by a one-way ANOVA. To test for any collection site effect, the Calabria region was divided in two principal areas (Tyrrhenian and Ionian) that are roughly separated by the mountains of Appennino Calabrese. Using the one-way ANOVA test, differences in contamination levels were evaluated between animals of the same species collected from the two areas. At least four samples were required for a species, and for a collection area within species, to be submitted to the test. All data were analyzed using SAS software (Statistical Analysis Systems Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

The analytical method was originally developed for the simultaneous detection of 18 OCs. However, the only compound detected in accurately measurable quantities was *p,p'*-DDE, with only traces quantities of the other compounds found. Since *p,p'*-DDE is higly stable, and is the main metabolite of the insecticide DDT, it is the more frequent OCs contaminant detected in wild bird tissues, and its concentrations are generally higher than those of the other compounds (Ford *et al.* 1990, Elliot *et al.* 1992, Newton *et al.* 1993, Jarman *et al.*, 1996, Kallenborn *et al.* 1998, Mateo *et al.* 2000). Consequently, *p,p'*-DDE can be considered a marker for OCs contamination in wild birds, and data regarding *p,p'*-DDE are particularly useful for comparing OCs contamination studies.

The compound *p,p'*-DDE was detected in all the analyzed liver samples. The majority of samples showed concentration in the hundreds of $\mu g kg^{-1}$ wet weight range. The calculated mean, geometric mean, minimum and maximum values for all species were 350, 210, 5 and 4470 $\mu g kg^{-1}$ (ppb) respectively. Data for each species are presented in Figure 2.

Species effects accounted for 25% of total variability, whereas collection site effects (Tyrrhenian and Ionian areas) accounted for less than 16% of total variability. Statistical analysis showed that the average contamination level was significantly greater in grey heron ($P<0.001$); whereas no

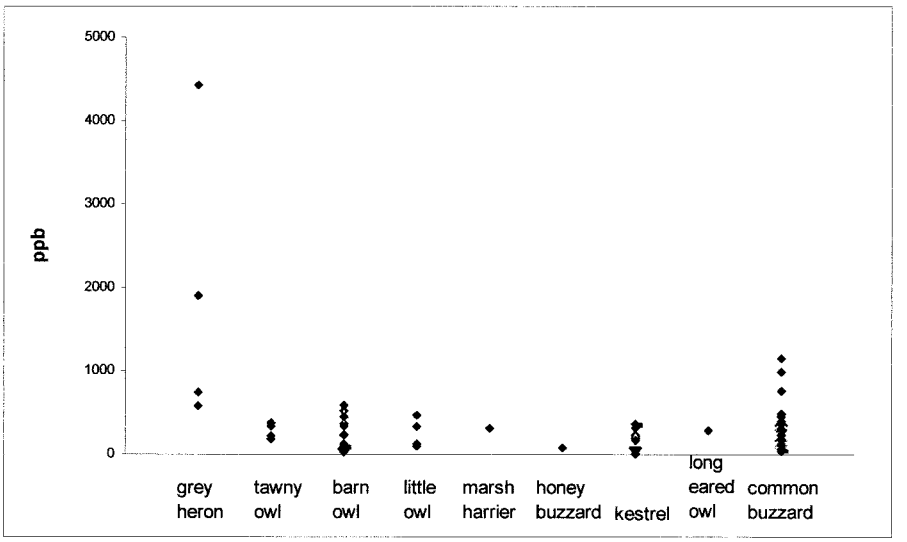

Figure 2. Levels of *p,p'*-DDE in liver of predatory birds (80 samples) collected from the territory of Calabria region.

significant differences were observed among the other monitored species (P>0.05), nor between birds collected from the Tyrrhenian and Ionian areas.

It should be noted that the grey heron is the only bird monitored in this study that feeds almost entirely on small fish and frogs, and it is considered a good indicator of OCs pollution (de Cruz *et al.*, 1997). Among predatory birds, fish eaters are particularly exposed to OCs contamination through the food chain. Thus, the greater contamination levels observed in grey heron may be attributed to its feeding habit. This, however, does not necessarily imply high contamination levels in fish given the large bioaccumulation capacity of fish-eating birds in relation to the levels present in the fish they feed upon. Fossi *et al.* (1995) say that this can be partially explained by the fact that lower evolved specialized feeder species do not have sophisticated enzyme systems designed to detoxify lipophilic compounds.

Habitats and/or home range did not seem to affect contamination levels since no significant differences were observed among the other monitored species. However, in a recent study conducted in Calabria region (Provini and Galassi, 1999) of eggs from two terrestrial (kestrel and sparrowhawk) and two aquatic (heron and mallard) types of birds, considerable DDT contamination (8,7 mg g^{-1} lipid) was observed in the kestrel eggs which was attributed to the fact that this bird lives close to villages. The data of the present study regarding a relatively large number of kestrel liver samples (15) showed only slight *p,p'*-DDE contamination (average 170

μgkg^{-1} wet weight); consequently, high contamination levels should only be found in connection with DDT point sources. In addition, no significant differences in contamination levels were observed between birds collected from the two different geographic areas (Tyrrhenian and Ionian). It should be noted, however, that the three species studied (barn owl, kestrel and common buzzard) are not sedentary, and hence, are not the more appropriate for comparative studies of contamination occurring in adjacent geographic areas.

In an analogous study conducted by Donato *et al.* (1996) of predatory birds collected from 1993 to 1994 in different areas of the Abruzzo region (central Italy) and the Campania region (southern Italy), 94% of the liver sample were positive for *p,p'*-DDE, with an average concentration of 450 ppb and a maximum value of 3970 ppb. These results agree well with those obtained in our study. On the whole, no observable differences between the three monitored regions were recorded, and contamination levels were largely lower than those known for inducing negative effects on the reproduction and dynamics of bird populations (McEwen and Stephenson, 1979).

Newton *et al.*(1993) monitored *p,p'*-DDE levels in the liver for an adequately large number of sparrowhawks, kestrels and herons in Britain that showed a progressive decline in contamination levels over about 30 years (1963-1990). The geometric mean values (expressed in $\mu g\ g^{-1}$ wet weight) for the last period they studied, 1986-1990, were 1.77 (sparrowhawk), 0.37 (kestrel) and 0.58 (heron). If the downward trend of *p,p'*-DDE levels (half-life of about 5 years) they reported is extrapolated to the period 1993-1997, contamination levels similar to those observed in the present study (Figure 2) result. These *p,p'*-DDE levels in raptor liver may be considered normal background levels for European countries during the 1990's, resulting from the uncontrolled use of DDT in the 1960's.

As already noted, the samples collected in the Calabria region were taken from birds found dead or dying due to reasons not always known. However, based on the levels of *p,p'*-DDE that have been recorded, DDT should be excluded as a cause of death. Future studies will probably also confirm the downward trend in OCs contamination in Calabria region, nevertheless, constant monitoring is still necessary to promptly indicate point pollution.

Acknowledgments. We thank the staff of "Comitato Italiano per la Protezione degli Uccelli Rapaci" and "Centro Recupero Animali Selvatici di Cosenza" for providing samples. We also thank Dr. Rosamaria Donato for her technical collaboration.

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