Bull Environ Contam Toxicol (2008) 80:497–501
DOI 10.1007/s00128-008-9457-y

Organochlorine Pesticide Concentrations in Sediment and Amphibian Tissue in Playa Wetlands in the Southern High Plains, USA

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Received: 21 November 2007 / Accepted: 24 April 2008 / Published online: 23 May 2008

Abstract Playa wetlands are critical habitat for wildlife in the Southern High Plains (SHP), a region dominated by agriculture. Little information on pesticide levels exists for playas, and thus we measured organochlorine pesticide concentrations in sediment and amphibians collected from playas in cropland and grassland watersheds. Heptachlor, α- and β-BHC, γ-chlordane, and dieldrin were detected in sediment and/or tissue samples, typically at or below 1 ng/g, dry weight. However, mean DDT and DDE reached 19.7 and 4.1 ng/g in sediments and 6.3 and 2.4 ng/g in tissues, respectively. Land use did not influence pesticide levels in sediment or amphibians.

Keywords *Bufo cognatus* · Great plains toad · Spadefoot toad · *Spea* spp.

Agricultural practices contribute to contaminant runoff into aquatic systems, with resulting effects on aquatic biota (Irwin et al. 1996; Thurman et al. 2000). The Southern High Plains (SHP) was once characterized by extensive short-grass prairie with an estimated 25,000 to 30,000 playa wetlands. Playas are shallow, depressional wetlands found in the SHP that generally dry each year (Smith 2003). Currently, cropland agriculture is the dominant use of land in the SHP, with cotton, sorghum, and wheat having replaced most of the native grassland (TDA 2001). In addition, erosion of topsoil has contributed to significant sedimentation of about 90% of the playas in the SHP, which reside at the lowest point in their respective watersheds (Samson and Knopf 1996). Interestingly however, little information is available on the level of pesticide deposition into playas.

Organochlorine pesticides (OC) are persistent compounds and widely distributed in the environment (Stickel 1968). Historically, γ-BHC and DDT have been used to control a variety of insect pests in crops in Texas (Leser et al. 1996). Specific OC use information is not readily available making it difficult to accurately predict the amount of OCs likely to be found in playas. However, some OC residue data are available for sediment, waterfowl, and ring-necked pheasants (*Phasianus colchicus*) residing in playas in the SHP (Wallace 1984; White and Krynitsky 1986; Flickinger and Krynitsky 1987; Irwin et al. 1996). Heptachlor epoxide and DDE were detected in ring-necked pheasant eggs (n =61), but while heptachlor epoxide was only detected in one egg, DDE was detected in 97% of the eggs (Wallace 1984). In waterfowl, endrin, heptachlor epoxide, and DDT and its metabolites DDE and DDD were detected in blue-winged teal (*Anas discors*) collected in spring and autumn, but only DDE and heptachlor epoxide were detected in mallards (*A. platyrhynchos*) (Wallace 1984). The average concentration of DDE in mallards was 0.62 ppm, wet weight (ww), which, Wallace (1984) noted, appeared high.

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Additionally, due to differences in concentrations between seasons, Wallace (1984) suggested that blue-winged teal were being exposed to OCs in wintering areas of Central and South America. In other studies of waterfowl in playas, sediment samples from feedlot-agriculture playas contained detectable concentrations of OCs (chlordane isomers; ≤0.22 ppm; Flickinger and Krynitsky 1987). Female northern pintail (*A. acuta*) and male green-winged teal (*A. crecca*) carcasses collected from feedlot playas had the highest concentration of DDE (1.2 ppm, ww) and heptachlor epoxide (9.3 ppm, ww), respectively, of any of the four species of ducks sampled (Flickinger and Krynitsky 1987). Irwin et al. (1996) only detected DDE in playa sediments (0.01–0.02 mg/kg).

Amphibians possess many life history traits that make them good indicators of contamination in aquatic and terrestrial environments (Collins and Storfer 2003). Anurans in general may accumulate contaminants (e.g. DDT) at a greater rate than other wildlife species (Meeks 1968). Diet, water regulation, trophic position, age, and the use of terrestrial and aquatic habitats during part or all of an amphibian's life affords many opportunities for contaminant exposure and resulting effects (Sparling 2000). Amphibians inhabiting the SHP rely on playas as the primary habitat for reproduction when precipitation and inundation of playas initiates breeding in spring (Stebbins 1951). Spring and summer generally correspond to application of pesticides to crops in the SHP when runoff of water and sediment from uplands should concentrate pesticides and other contaminants in cropland playas (Thurman et al. 2000). However, this assumption has never been tested and thus we examined OC concentrations in sediments and select amphibian species collected from cropland and native grassland playas. We hypothesized that OC concentrations in sediment and selected amphibians collected from playas in cropland-dominated watersheds would be greater than from playas in grassland-dominated watersheds.

Materials and Methods

Study playas were at least 0.8 km apart, and categorized as either cropland or grassland if more than 75% of their respective watersheds were either in row crop agriculture or native grassland, respectively. One sediment sample was collected from each of six cropland and six grassland playas (n = 12) located within 22 km of each other in Floyd County, TX, USA (Latitude: N 33°56′, Longitude: W 101°10′). Sediment also was collected in Deaf Smith County, TX, USA (Latitude: N 34°15′, Longitude: W 102°50′) from three playas embedded in a more contiguous grassland-area (across multiple watersheds) compared to Floyd County. Thus, these playas were not considered "grassland islands" like the grassland playas in Floyd County. Deaf Smith playas were treated separately from other grassland playas throughout analyses. All sediment samples were collected in 2003 from the top 10 cm strata and stored in 500-mL chemically clean glass jars (washed, and rinsed with acetone and hexane). Organic carbon content typically ranges between 1.2% and 1.7% in the top 15 cm of playas and decreases with depth (Luo 1994). Because substantial mixing of sediments occurs in playas due to wind and wave action (Smith 2003), we assumed that one sediment sample was representative of concentrations in sediment throughout the playa.

Recently emerged *Spea* spp. from two cropland and four grassland playas (n = 6 playas) and *B. cognatus* from four cropland and three grassland playas (n = 7 playas; all in Floyd County) were collected opportunistically concurrent with sediment samples. *Spea multiplicata* and *S. bombifrons* are included in the *Spea* spp. complex as they are difficult to distinguish as recent metamorphs (Stebbins 1951). Metamorphs were collected from the edge of the playa, euthanized using tricaine methane sulfonate (MS-222), and stored in Whirl-pak bags on ice for transport to the laboratory.

All sediment and tissue samples were stored at −20°C and processed within 15 months of collection. Sixty-three *Spea* spp. and 47 *B. cognatus* metamorphs were collected from the 13 playas. Six to 15 *Spea* spp. metamorphs (5.06–8.02 g, composite wet weight) and 4 to 9 *B. cognatus* metamorphs (4.81–9.46 g, composite wet weight) were pooled from each playa. Composited amphibian samples were dried overnight at room temperature until dry. Sediment samples were dried at room temperature to a constant mass and homogenized by hand grinding with a mortar and pestle. Extraneous organic matter (e.g. grass, roots) was removed during grinding and sifting (mesh size = 2 mm). Samples of sediment (approximately 10 g) from each playa were weighed. Approximately 20 g of Na_2SO_4 and 5–12 times the dry weight of the sample was added to sediment and amphibian samples, respectively. One sediment sample and either 1 pooled *B. cognatus* sample or 1 pooled *Spea* spp. sample were analyzed per playa (two samples per playa), with the exception of one playa with two amphibian samples, one from each species (three samples for this playa). All samples were spiked with 2 μL of internal standards of tetrachloro-*m*-xylene (TCMX) and decachlorobiphenyl (DCBP) in acetone to determine extraction efficiency. Based on recoveries of the internal standards, the extraction procedure was considered quantitative (>90%), therefore sample concentrations of OCs were not adjusted for extraction efficiency. Two samples of Ottawa sand (mesh size = 20–30; Fisher Scientific Co., Pittsburg, PA) were used as extraction method blanks, one for sediment and the other for tissue. Sample batches were extracted separately using a Dionex 200 accelerated solvent extractor (Sunnyvale, CA) with hexane:acetone (50%:50%) under the following parameters: pressure = 1300 psi, temperature = 100°C, extraction time = 14 min. Samples were rotary evaporated, brought to volume (2 mL), and transferred into autosample vials.

Two sets of organochlorine standards were prepared, each at concentrations of 0.1, 0.2, 0.5, 1, 2, 5, 10, and 100 µg/L (CLP-023R-40X and CLP-024R-40X; AccuStandard, New Haven, CT). In total, the two standards contained α-, β-, δ-, and γ-BHC, DDT, DDD, DDE, dieldrin, endrin, endrin aldehyde, endrin ketone, aldrin, heptachlor, heptachlor epoxide (isomer B), methoxychlor, endosulfan I and II, endosulfan sulfate, α- and γ-chlordane, TCMX, and DCBP. Samples and standards were analyzed using a gas chromatograph (Hewlett Packard 6890 series) with an electron capture detector, using a DB5-MS column (30.0 m × 250 µm × 0.25 µm, J&W Scientific). An inlet in splitless mode was used with helium gas flowing at 89.0 mL/min at a pressure of 33.29 psi. Purge time was 0.50 min at 80.0 mL/min. Inlet and detector temperatures were 225°C and 280°C, respectively. The temperature program started at 100°C with a 1 min equilibration time, was then increased at 4°C/min to 240°C, held for 1 min at 240°C, and then increased at 8°C/min to 270°C, and finally held at 270°C for 3 min for a program length of 42.75 min. Residue concentrations were calculated based on matching retention times of sample peaks with those of the calibration standards. We used a GC coupled to a mass spectrometer (MS) in selected ion mode to confirm identity of OCs in samples. In most instances, sample concentrations were too low to allow for MS confirmation. The limit of quantification for sediment was 0.02 ng/g based on the concentration of the lowest standard in the calibration curve, the use of 10 g of sediment, and concentration of the sediment extract to 2 mL.

Statistical analyses were limited due to small sample size and the number of detects. For sediment, one-half the detection limit was substituted for non-detects where at least half the samples per land use had detectable concentrations of the compound (Table 1). Non-detects that were replaced by one-half the detection limit were DDE in cropland (n = 1) and Deaf Smith County grassland (n = 1) samples and dieldrin in cropland samples (n = 3). Sediment OC concentrations were tested for normality for each land use with sufficient samples (i.e., DDT, DDE, and dieldrin) using R (R 2005). Differences between land uses of these OC concentrations in sediment were tested with a one-way ANOVA. The remaining sediment samples and all tissue samples were reported as ranges of detected concentrations since the number of non-detects was generally greater than detected concentrations which precluded statistical analyses.

Results and Discussion

Twenty OCs were included in the two standard mixes used in this study. Seven of these compounds were detected (more than once) in sediment and tissue samples. Six OCs were detected at least once in sediment (Table 1) and four in tissue samples (Table 2). DDT, DDE, and heptachlor were the only compounds detected in both matrices.

Concentrations of DDT, DDE, and dieldrin in sediments did not differ ($p \geq 0.182$) between cropland and grassland playas (Table 1). Generally, α-BHC, heptachlor, and γ-

Table 1 Mean ± SE (ng/g, dry weight) of organochlorine (OC) concentrations found in sediment collected from playas located in different land use types in the Southern High Plains in 2003

OC	Cropland	n[a]	FC[b]	n[a]	DSC[b]	n[a]	F[c]	*p*
N[d]		6		6		3		
DDT	14.6 ± 3.9	6	19.7 ± 4.4	6	13.1 ± 1.9	3	0.667	0.531
DDE	4.1 ± 2.6	5	2.0 ± 0.9	6	0.5 ± 0.1	2	1.67	0.230
Dieldrin	0.6 ± 0.03	3	0.7 ± 0.03	6	1.2 ± 0.6	3	1.97	0.182
α-BHC	(0.4–0.6)[e]	2	(0.4–0.6)	2	1.1 ± 0.3	3	–[f]	–[f]
Heptachlor	(0.3–0.4)	2	(0.3–0.4)	2	<0.5[g]	0	–[f]	–[f]
γ-Chlordane	(0.4)	2	(0.2–0.3)	2	<0.4[g]	0	–[f]	–[f]

[a] Column is number of detects for each OC

[b] FC = Floyd County – patchy grassland areas, DSC = Deaf Smith County – contiguous grassland areas

[c] Degrees of freedom are 2, 12 for statistical analyses

[d] Number of samples analyzed for each land use type

[e] Range of detects for the compound and land use type in parentheses

[f] Not applicable due to sample size in each land use for each compound

[g] Calculation is based on mean sample mass (within each land use type) and one-half the detection limit

Table 2 Range (ng/g, wet weight) of organochlorine (OC) concentrations found in whole body tissue of *Spea* spp. and *Bufo cognatus* metamorphs collected from playas located in two different land use types in Floyd County, Texas in 2003

OC	*Spea* spp.				*B. cognatus*			
	Cropland	n[a]	Grassland	n[a]	Cropland	n[a]	Grassland	n[a]
N[b]		2		4		4		3
β-BHC	<0.5[c]	0	0.7[d]	1	<1.3	0	2.1	1
DDT	2.4	1	3.0–6.3	4	3.2	1	4.2, 6.3	2
DDE	1.0, 1.5	2	1.0	1	0.8–2.4	4	0.4, 0.5	2
Heptachlor	<0.3	0	<0.3	0	0.4–1.0	3	<0.6	0

[a] Column is number of detects for each OC

[b] Number of samples analyzed for each land use type

[c] Calculation is based on mean sample mass (within each species and land use type) and one-half the detection limit

[d] Concentration of compound in a single sample

chlordane were detected in only a portion of the playas within each land use type. Concentrations of α-BHC, heptachlor, and γ-chlordane were usually near detection limits, with maximum mean concentrations of only about 1 ng/g for α-BHC and dieldrin in Deaf Smith County playas (Table 1). DDT and DDE were detected in nearly 100% of the samples, typically at concentrations several fold higher than the other OCs (Table 1). In addition, mean concentration of DDT was several fold greater than DDE for all land use types (Table 1).

Greater historical use of DDT in Texas compared to other compounds (Leser et al. 1996) could account for the greater concentrations of DDT and DDE relative to other OCs. Typically, concentrations of DDE would be expected to exceed DDT in environmental samples due to degradation of the parent compound following a long period of non-use (Hunt et al. 1986). Yet, ratios of DDT to DDE in our sediment samples are suggestive of current and/or recent use of DDT in some capacity that facilitates deposition of DDT in the SHP. However, recent studies suggest that lower DDE/DDT ratios may indicate historical heavy use and long persistence of DDT as opposed to recent use (Mora 1997). Records of use in the SHP of DDT and other OCs are limited (mosquito control, Guerrant et al. 1970; cotton pest control, Leser et al. 1996), thus it is difficult to estimate the magnitude of use of these compounds. Alternately, persistent OCs could mix between cropland and grassland playas as a result of atmospheric deposition of OCs across the landscape. Even if OC distribution was originally weighted toward cropland watersheds, eolian deposition of soil (including global sources) could result in significant redistribution of persistent pesticides onto grassland watersheds.

Amphibians did not appear to accumulate OCs to any significant degree in this study. DDT, DDE, β-BHC, and heptachlor were the only OCs regularly detected in amphibians, and overall, concentrations of DDT and DDE in amphibian tissue (wet weight) were lower than sediment concentrations (dry weight) (Table 2). Yet relative to the other OCs, concentrations and frequency of detection of DDT and DDE in amphibian samples did parallel the relative DDT and DDE contributions from sediments (Table 1). However, comparisons of OC concentrations between land uses for both amphibian species were not possible given the paucity of detects.

Mean concentrations of DDE in *Spea* spp. and *B. cognatus* were more than 80 times higher in our study than the maximum mean concentration (0.01 ng/g DDE) determined in pooled samples of juvenile green frogs (*Rana clamitans*) sampled in Michigan (Gillilland et al. 2001). Gillilland et al. (2001) did not attribute any effects such as deformities to concentrations of OCs detected. Dosing concentrations of DDT (*R. temporaria* dosed for 24 h with 5 ppb DDT) below those detected in sediment (potential source of a dose) in this study have been shown to cause behavioral effects (i.e., "frantic" movements) in tadpoles (Cooke 1972). However, concentrations determined in tissues of *Spea* spp. and *B. cognatus* were below levels found to cause mortality or developmental effects in tadpoles of many species (Sparling 2000).

Sensitivity of amphibians to OCs varies among species and developmental stages. However, from this study, no conclusions can be drawn about effects on tadpoles or adults exposed to the concentrations detected because these life stages were not analyzed. Sanders (1970) showed that OC toxicity varied among species, although age was not the same between species compared. Similarly, Cooke (1972) found Bufonids to be more tolerant to DDT than Ranids during larval development, although the Bufonids accumulated higher concentrations of pesticides. Similarly, older *B. woodhousii* tadpoles were more sensitive to DDT than younger tadpoles in tests of tolerance over the same exposure duration (Sanders 1970). This also has been noted in other species at different life stages (Sparling 2000). In addition to sensitivity, Ferguson et al. (1967) determined that amphibian populations inhabiting water bodies that were likely exposed previously to OCs had much greater tolerances to particular compounds (e.g. dieldrin, aldrin, DDT) than unexposed populations, a phenomenon that could also occur in playa amphibians. Additionally, Cooke (1972) noted that as tadpoles increased in size, they appeared to be removing increasing amounts of DDT from the dosing solution. Although DDT is known to biomagnify in the foodchain, amphibians we collected did not appear to be bioaccumulating DDT since tissue OC concentrations based on wet weight were lower than sediment OC concentrations (dry weight).

Amphibians inhabiting playas in the SHP are exposed to low concentrations of some OCs. Given that sediment concentrations of OCs were not different between land uses, OCs do not appear to be concentrated in cropland playas, possibly due to a number of environmental and/or anthropogenic factors. Differences of OC concentrations between land uses in whole body tissue of either amphibian species could not be determined, in part due to small sample size and concentrations near or below detection limits. Concentrations of OCs detected in sediment and amphibian tissues were generally below known effects concentrations, however, no definitive conclusions about effects can be made because toxicity was not assessed in this study. Further studies are needed to determine how OCs are entering native grassland playas. Additionally, development of resistance to OCs, as well as other compounds, should be studied in these amphibian species as they appear to be chronically exposed to persistent compounds such as OCs.

Acknowledgments This study was funded by Environmental Protection Agency project R-82964101-0 and National Science Foundation project DMS-0201105. We thank the numerous landowners who granted us access to field sites. We thank Xiaoping Pan, Kang Tian, and George P. Cobb for help with chemical analyses.

References

Collins JP, Storfer A (2003) Global amphibian declines: sorting the hypotheses. Divers Distrib 9:89–98. doi:10.1046/j.1472-4642.2003.00012.x

Cooke AS (1972) The effects of DDT, dieldrin and 2,4-D on amphibian spawn and tadpoles. Environ Pollut 3:51–68. doi:10.1016/0013-9327(72)90017-1

Ferguson DE, Chapman Gilbert C (1967) Tolerances of three species of anuran amphibians to five chlorinated hydrocarbon insecticides. J Miss Acad Sci 13:135–138

Flickinger EL, Krynitsky AJ (1987) Organochlorine residues in ducks on playa lakes of the Texas panhandle and eastern New Mexico. J Wildl Dis 23:165–168

Gillilland CD, Summer CL, Gilliland MG, Kannan K, Villeneuve DL, Coady KK, Muzzall P, Mehne C, Giesy JP (2001) Organochlorine insecticides, polychlorinated biphenyls, and metals in water, sediment, and green frogs from southwestern Michigan. Chemosphere 44:327–339. doi:10.1016/S0045-6535(00)00335-0

Guerrant GO, Fetzer LE Jr, Miles JW (1970) Pesticide residues in Hale County, TX, before and after ultra-low volume aerial application of malathion. Pestic Monit J 4:14–20

Hunt WG, Johnson BS, Thelander CG, Walton BJ, Risebrough RW, Jarman WM, Springer AM, Monk JG, Walker W II (1986) Environmental levels of p,p′-DDE indicate multiple sources. Environ Toxicol Chem 5:21–27. doi:10.1897/1552-8618(1986)5[21:ELOPIM]2.0.CO;2

Irwin RJ, Connor PJ, Baker D, Dodson S, Littlefield C (1996) Playa lakes of the Texas high plains: a contaminants survey and assessment of biological integrity. USFWS, Ecological Services Field Office, Arlington, Texas

Leser JF, Karner MA, Ward CR, Walker JK (1996) Insect and mite pest management in the Southwest. In: King EG, Phillips JR, Coleman RJ (eds) Cotton insects and mites: characterization and management. The Cotton Foundation Publisher, Memphis, pp 695–739

Luo HR (1994) The effects of land use on sediment deposition in playas. Master's thesis. Texas Tech University, Lubbock, TX

Meeks RL (1968) The accumulation of 36Cl ring-labeled DDT in a freshwater marsh. J Wildl Manage 32:376–398. doi:10.2307/3798983

Mora MA (1997) Transboundary pollution: persistent organochlorine pesticides in migrant birds of the southwestern United States and Mexico. Environ Toxicol Chem 16:3–11. doi:10.1897/1551-5028(1997)016<0003:TPPOPI>2.3.CO;2

R Development Core Team (2005) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN: 3-900051-07-0. URL http://www.R-project.org. Accessed 16 May 2008

Samson FB, Knopf FL (1996) Prairie conservation: preserving North America's most endangered ecosystem. Island Press, Washington, DC, 339 pp

Sanders HO (1970) Pesticide toxicities to tadpoles of the western chorus frog *Pseudacris triseriata* and Fowler's toad *Bufo woodhousii fowleri*. Copeia 1970:246–251. doi:10.2307/1441646

Smith LM (2003) Playas of the great plains, 1st edn. University of Texas Press, Austin

Sparling DW (2000) Ecotoxicology of organic contaminants to amphibians. In: Sparling DW, Linder G, Bishop CA (eds) Ecotoxicology of amphibians and reptiles. Society of Environmental Toxicology and Chemistry, Pensacola, pp 461–494

Stebbins RC (1951) Amphibians of western north America. University of California Press, Berkeley and Los Angeles

Stickel LF (1968) Organochlorine pesticides in the environment. US Department of the Interior, Washington, DC

Texas Department of Agriculture (TDA) (2001) Texas agricultural statistics 2000. Texas Agricultural Statistics Service, Austin

Thurman EM, Bastian KC, Mollhagen T (2000) Occurrence of cotton herbicides and insecticides in playa lakes of the High Plains of west Texas. Sci Total Environ 248:189–200. doi:10.1016/S0048-9697(99)00542-2

Wallace BM (1984) Organochlorine pesticide residues in pheasant eggs and waterfowl from the Texas High Plains. Master's thesis. Texas Tech University, Lubbock, TX

White DH, Krynitsky AJ (1986) Wildlife in some areas of New Mexico and Texas accumulate elevated DDE residues, 1983. Arch Environ Contam Toxicol 15:149–157. doi:10.1007/BF01059964