DDT exposure of frogs: a case study from Limpopo Province, South Africa

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Highlights

- Contaminants is suspected to play a part in amphibian declines worldwide
- No POPs data in frogs from areas where DDT is used for malaria control
- Quantifiable DDT was found in frog fat from an area where DDT is used
- No testicular oocytes were found in any frog
- Significant asymmetric testicular morphology in frogs from the sprayed area

Abstract

Amphibians are globally under pressure with environmental contaminants contributing to this. Despite caution aired more than 80 years ago of threats posed to amphibians by DDT spraying for disease vector control, no data have been published on concentrations or effects of DDT contamination in frogs from areas where DDT is actively sprayed to control the insect vectors of malaria. In this study, we sampled fat bodies of *Xenopus laevis and X. muelleri* naturally occurring in an area where indoor residual spraying of DDT is employed and from adjacent, non-sprayed, areas. ΣDDT concentrations ranged between <LOQ and 280 ng/g ww (wet weight) from the non-sprayed area, and 5.5 to 910 ng/g ww from the sprayed area, but statistical significance could not be shown. We observed significant asymmetric testicular morphology in frogs from the sprayed area, possibly due to endocrine disruption by compounds such as the DDTs. A previous study from the same area found very high concentrations of DDT in the eggs of the Grey Heron *Ardea cinerea*. This suggests that the DDT we found in frogs may have contributed to DDT loadings higher in the food

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web. These findings, combined with other studies from this area, support the need to reduce and eventually move away from DDT in malaria control safely and sustainably.

Key words: *Xenopus*, DDT, South Africa, Amphibia, Endocrine disruptive compounds, Fluctuating asymmetry

1. Introduction

With amphibians globally under pressure (Blaustein et al. 2011; Collins and Storfer 2003), chemical contaminants may be contributing to their decline (Carey and Bryant 1995). DDT has been used since the late 1940's to control mosquitoes and other disease vectors in South Africa, but DDT use is now globally restricted by the Stockholm Convention (Bouwman 2003; van Dyk et al. 2010). To achieve control, DDT is applied at 2 g/m^2 on indoor walls and other surfaces of residences for malaria control, thereby interrupting transmission of the parasite by the vector. From decades of use, residues of DDT associated with malaria control have accumulated in the environment and in human and animal tissues. DDT residues have been measured in human milk (Bouwman et al. 1990, Bouwman et al. 2006, Sereda et al. 2009) and blood (Aneck-Hahn et al., 2007, Whitworth et al., 2014; Gaspar et al. 2015) from inhabitants to the east of Thohoyandou, Vhembe District, Limpopo Province (Fig. 1). Terrestrial and aquatic bird eggs from the same area contained high concentrations of DDT; e.g. Grey Heron *Ardea cinerea* eggs had the highest ΣDDT concentrations reported this century for any aquatic bird worldwide (Bouwman et al. 2013). No breeding colonies of water birds could be found in the sprayed areas between Thohoyandou and the Kruger National Park (KNP), despite repeated and extensive searches. It was speculated that reproductive failure might have caused local disappearance, similar to what was found in Canada after similar concentrations of DDT occurred in Great Blue Heron eggs *Ardea herodias* (Laporte 1982). Eggshells of the terrestrially feeding Cattle Egret *Bubulcus ibis* from the DDT spayed area were significantly thinner and had significantly higher concentrations of DDT (Bouwman et al. 2013). Fish from the Luvuvhu River contained DDT and the levels were particularly higher inside the sprayed area (Barnhoorn et al. 2009). Very high concentrations of ΣDDT (mean 4 249, max 22 000 ng/g lipid) were found in Tigerfish *Hydrocynus vittatus* from the Luvuvhu River inside the KNP, downstream of the DDT-sprayed area (Gerber et al. 2016).

Food items of the Grey Heron (an aquatic predator) include fish and amphibians (Hockey et al. 2005). Although there are published studies on DDT in fish from sprayed areas (e.g. Barnhoorn et al. 2009; Bouwman et al. 1990), there is very little data from Africa about DDT in amphibians. DDT residues were detected in frogs from Somalia in relation to a pesticide spill (Lambert 1997), and DDT was quantified in frogs from the Rift Valley (Lincer et al. 1981). With the high concentrations of DDT found in fish and bird eggs from Limpopo, the residue concentrations in frogs should be investigated, given the sensitivity of amphibians to toxic and endocrine disrupting (ED) pollutants (Carey and Bryant 1995) and as a possible vector of DDT to higher trophic level animals. It is interesting to note that the first caution about the potential impacts on amphibians of DDT used for malaria control was published in Science in 1944 (Ellis et al. 1944). However, more than 80 years later, we could find no reference to DDT in frogs from any area where DDT is actively used for malaria control. This study aimed at quantifying and interpreting DDT and metabolite concentrations and somatic

parameters in frogs from an area where DDT is currently used for malaria vector control compared with a reference area where DDT is not used.

Figure 1: Map of the study area with sample locations indicated. The DDT-sprayed area is east of the city of Thohoyandou. + Sites in reference area • Sites in DDT-sprayed area

2. Materials and methods

2.1 Ethical clearance and permits

This study was approved by the North-West University Ethics Committee (NWU-0053-08-A6). Permission for collection was granted by the Department of Economic Development, Environment and Tourism: Limpopo Province (CPM001958). This permit allowed for the collection of no more than 50 specimens per species from the study area.

2.2 Sampling

We collected African Clawed Frogs *Xenopus laevis* and Müllers Platanna *Xenopus muelleri* from ponds and small dams in Limpopo Province where DDT is used for malaria control (Fig. 1) and sampled their fat bodies. The collection campaign lasted 10 days in December 2009 using baited funnel traps, deploying three to four traps per site at multiple sites per night for 12 hours per trap. The traps were baited in the afternoon and left overnight to be collected the following morning. The frogs were transported in containers with water

from their habitat back to base camp. Each frog was anaesthetised in an MS 222 (tricaine methanesulfonate) solution, weighed, and measured. Testes dimensions were measured in the field on fresh frogs as measurement units (mu) using a graduated ocular in a dissection microscope – real scale measurement was not possible at the time. Fat bodies were collected from each frog, weighed, and frozen in foil (foil was prepared by washing three times respectively with ethanol and hexane).

2.3 Chemical analyses

Of the 38 frogs sampled, 22 were analysed for *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD, and *o,p'*-DDD. DDT concentrations were analysed by an ISO 17025 accredited laboratory (Food and Drug Assurance Laboratories, Pretoria) using the European standard method NF-EN-1528 in fatty foodstuffs, using GC-MS. A C18 cartridge was used to extract DDT and its metabolites, followed by a florisil cartridge clean up according to Bordet et al. (2002). The analytes were eluted with a petroleum ether – diethyl ether mixture. Since Aldrin was not detected in unfortified samples, it was used as an internal standard. Quantification was via fortified calibration curves in matrix; the correlation coefficient was 0.99. Limits of detection (LOD) was three times the standard deviation of background noise, and limits of quantification (LOQ) was three times the LOD. The LOQs were 15 ng/g wet weight (ww) for *p,p'*-DDT, 10 ng/g ww for *p,p'*-DDE and *o,p'*-DDT, 5 ng/g for ww *p,p'*-DDD and *o,p'*-DDD, and 1 ng/g ww for *o,p'*-DDE. Means were calculated for positive samples only.

2.4 Histological preparations

To assess possible endocrine disruption, histological preparations were made of testes from eight randomly selected male frogs (four from DDT-sprayed and four from non-sprayed areas), and screened for testicular oocytes. The sample from the DDT-sprayed area consisted of two *X. muelleri* from two different sites, and two *X. laevis* from one site. The sample for the non-DDT-sprayed area consisted of one *X. muelleri* and three *X. laevis* from three different sites. Preparation of the histology slides was adapted from Weldon (2005) and Jooste et al. (2005). Serial sections were made longitudinally at 6 µm intervals and then stretched and affixed to microscope slides using Mayer's albumen and a heating plate (45 ºC). The slides were then dried overnight at 35 ºC. The slides were coloured with Mayer's haematoxylin and eosin, and fixed. The fixed slides were inspected under a light microscope (Nikon YS100).

2.5 Statistical analyses

Statistical analyses and visual representation of results were done with GraphPad Prism Version 4 for Windows (GraphPad Software, Inc., CA, USA). Morphometric data were compared between the reference and study areas using two-way, unpaired, Mann Whitney non-parametric tests. Comparisons of the left and right testes within the reference or study areas were done with a paired T-test.
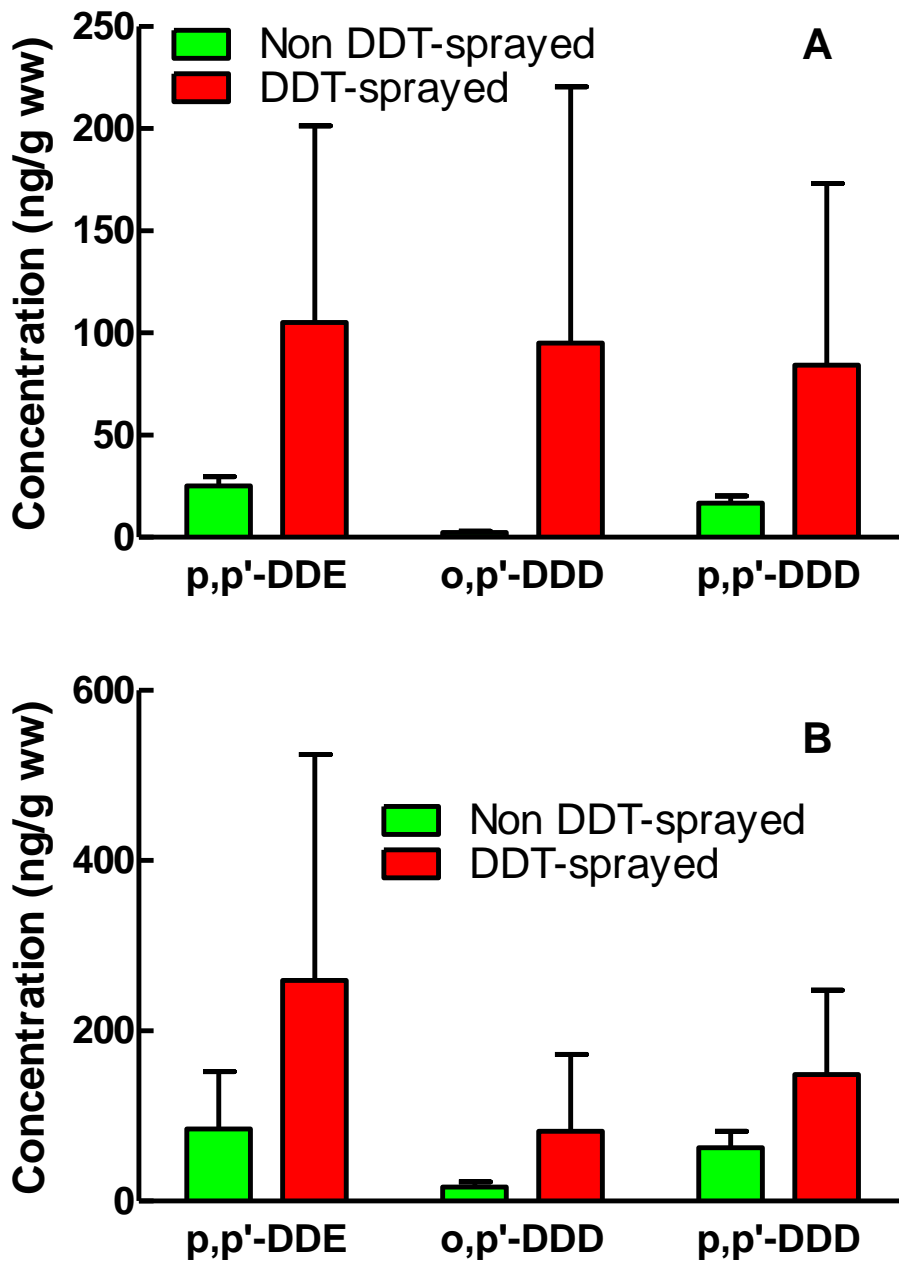
Figure 2: Means and standard deviations of *p,p'*-DDE, *o,p'*-DDD and *p,p'*-DDD in frog fat from the Limpopo Province. Due to low sample numbers, further statistical comparisons were not possible. A – Male frogs; B - Female frogs.

3. Results

Combined, we conducted 25 trap nights (multiple sites per night over 10 days) with approximately 2400 combined trap hours. The sites were located between 150-400 m away from the closest river, all of them flowing only sporadically after rains, except Site 17 that was 400 m away from the permanently flowing Luvuvhu River. Ten sites were visited with

only five of these (Fig. 1) yielding *Xenopus* frogs. We collected 38 specimens of the two *Xenopus* species from both areas combined.

Individual results and means of residue concentrations are presented in Table 1. Neither *p,p'*-DDT nor *o,p'*-DDT could be quantified in any sample. All but one frog (no. 7 in Table 1) had quantifiable residues for at least one of the DDT metabolites. There were not enough data to conduct statistical comparisons on the residue concentrations. The differences in concentrations for male and female *Xenopus* species are graphically presented in Fig. 2.

Table 1: DDT concentrations (ng/g wet weight) in the fat bodies of *Xenopus* sp. caught in DDT-sprayed and non-DDT-sprayed areas. *o,p'*-DDT and *p,p'*-DDT was not quantifiable in any sample.

Site	No.	Species	Sex	Area	*o,p'*-DDE	*p,p'*-DDE	*o,p'*-DDD	*p,p'*-DDD	ΣDDT
36	5	X. muelleri	male	Non-DDT sprayed	<LOQ	19.8	4.5	12.6	36.9
36	6	X. laevis	male	Non-DDT sprayed	<LOQ	12.1	1.3	5.7	19.1
36	7	X. muelleri	male	Non-DDT sprayed	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
36	8	X. laevis	male	Non-DDT sprayed	<LOQ	15.6	1.3	22.6	39.5
36	10	X. laevis	male	Non-DDT sprayed	<LOQ	21.9	<LOQ	7.6	29.5
36	12	X. laevis	male	Non-DDT sprayed	<LOQ	42.0	<LOQ	10.9	52.9
36	13	X. laevis	male	Non-DDT sprayed	<LOQ	48.8	0.9	36.8	86.5
36	16	X. laevis	male	Non-DDT sprayed	<LOQ	20.9	3.2	21.7	45.8
35	21	X. laevis	male	Non-DDT sprayed	<LOQ	20.2	3.3	15.5	39.0
35	25	X. muelleri	male	Non-DDT sprayed	<LOQ	<LOQ	<LOQ	6.6	6.6
				MEAN		**25.2**	**2.4**	**15.6**	**39.5**
37	28	X. laevis	male	DDT-sprayed	<LOQ	39.4	6.6	52.9	98.9
37	30	X. laevis	male	DDT-sprayed	<LOQ	<LOQ	<LOQ	5.5	5.5
37	35	X. laevis	male	DDT-sprayed	<LOQ	53.5	<LOQ	25.6	79.1
37	37	X. muelleri	male	DDT-sprayed	<LOQ	80.6	<LOQ	110.0	190.6
5	40	X. muelleri	male	DDT-sprayed	<LOQ	247.2	183.8	227.1	658.1
				MEAN		**105.2**	**95.2**	**84.2**	**206.4**
35	20	X. laevis	female	Non-DDT sprayed	18.9	17.7	22.3	42.5	101.4
35	24	X. laevis	female	Non-DDT sprayed	<LOQ	152.0	23.1	102	277.1
35	26	X. muelleri	female	Non-DDT sprayed	<LOQ	119.2	4.8	43.1	167.1
17	4	X. muelleri	female	Non-DDT sprayed	<LOQ	<LOQ	0.8	11.8	12.6
				MEAN	**18.9**	**96.3**	**12.8**	**49.9**	**139.6**
37	33	X. laevis	female	DDT-sprayed	46.3	477.1	145.9	245.1	914.4
37	39	X. laevis	female	DDT-sprayed	<LOQ	70.9	18.3	47.8	137.0
5	41	X. muelleri	female	DDT-sprayed	<LOQ	<LOQ	<LOQ	154.8	154.8
				MEAN	**46.3**	**274.0**	**82.1**	**149.2**	**402.1**

Statistical analysis could only be done with the morphometrics of male *X. laevis* as there was not enough *X. muelleri* (Table 2). None of the morphometric measurements were

Table 2: The mean morphometric data obtained from all the collected *Xenopus* specimens.

Area	Species	n*	Mass (g)	Length (mm)	Head width (mm)	Right testes length (mu**)	Right testes width (mu)	Left testes length (mu)	Left testes width (mu)	Left liver mass (g)	Heart mass (g)	Fat body mass (g)	Egg mass (g)
Males													
Non DDT-sprayed	*X. muelleri*	3	47.1	65.3	23.7	86	34	89	32	0.72	0.17	0.82	
	X. laevis	17	29.9	59.2	20.0	81	33	83	31	0.69	0.19	0.48	
DDT-sprayed	*X. muelleri*	2	24.0	57.5	17.8	76	28	77	28	0.63	0.23	0.20	
	X. laevis	9	30.4	60.3	20.2	86	29	91	28	0.77	0.18	0.51	
Females													
Non DDT-sprayed	*X. muelleri*	1	27.7	56.8	18.4					0.75	0.15	0.25	1.00
	X. laevis	2	44.7	76.5	22.6					0.88	0.18	0.38	2.60
DDT-sprayed	*X. muelleri*	2	32.9	64.2	20.1					1.05	0.13	0.38	2.40
	X. laevis	2	37.4	68.0	20.5					1.70	0.40	0.43	3.08

*Numbers of frogs collected not representative of sex distribution as number restrictions on permit only limited us to 50 individuals per species and a greater proportion of male frogs were selected for dissection
** Measurement units.

significantly different between the two areas (p values ranged between 0.4379 – 0.8608). However, the mean width of the right testes of males from the DDT-sprayed area was significantly (p = 0.0252) smaller than the mean of the right testes from the non-sprayed area. Additionally, measurements were compared between the left and right testes within the respective study areas. The right testes of the males from the DDT-sprayed area were significantly shorter (p = 0.0447) than their left testes. The widths of their right and left testes did not differ significantly. Neither the testes width nor the length differed significantly between right and left for the frogs from the reference area.

One *X. laevis* from the DDT-sprayed area had an extra left testis; this additional testis was smaller (length ~16%, width 60%) than the primary left testis. The right testis appeared normal. None of the screened testes had any testicular oocytes.

4. Discussion

DDT-derived compounds had quantifiable concentrations of DDT and metabolites in frogs from both areas (Table 1). Despite the inability to compare statistically, generally higher concentrations in the DDT-sprayed areas were observed (Fig. 2). The DDTs are most likely derived from malaria control operations as there has never been commercial farming in the DDT-sprayed area, even when DDT use was still allowed for agriculture in SA. [DDT was banned in South Africa for agricultural purposes in 1976, with use of existing stocks prohibited after 1983 (Bouwman 2003)].

Due to the long distances between the sample sites and because the sites where they were collected are not directly connected by rivers or streams (Fig. 1), it is unlikely (but not impossible) that the DDT detected in frogs from the non-sprayed areas (all located higher up in the catchment) were due to frogs immigrating upstream and overland from the DDT-sprayed areas. Furthermore, many of the collection sites were isolated ponds, suggesting that the DDT came from local runoff. In a situation of colonisation after release in a temperate area (France), dispersal through water bodies was much faster than overland (Fouquet and Measey 2006). Since the ponds we collected the frogs were isolated and not connected to other streams, wetlands, or rivers, the residues in the reference area may therefore be a legacy from long-range transport from sprayed areas (as also noted by Fellers et al. 2004 and Sparling et al. 2001), past use, illegal current use, or a combination thereof. We have previously detected high concentrations of DDT in sparrow eggs from a town located outside the sprayed area (Bouwman et al. 2013) which we also ascribed to possible illegal DDT use.

Although the female frogs were generally larger than the males, their fat bodies were smaller than those of the males (Table 2). Females however, had higher concentrations of DDT metabolites (Fig. 2). We cannot explain this difference, as one would expect that egg deposition would reduce the overall load of DDT in the female. Concentrations of both *p,p'*-DDT and *o,p'*-DDT could not be quantified in any frog, possibly due a relatively high LOQ. A lower analytical LOD could have improved the interpretability of the results. However, the percentage of *p,p'*-DDT (of the sum of all DDTs measured) was also low (min 0.58%, mean 0.97%, max 1.2%) in Grey Heron eggs from the same area (Bouwman et al. 2013), indicating a very low percentage of *p,p'*-DDT in the aquatic environment. Jofré et al. (2008) also found no quantifiable *p,p'*-DDT in frogs from Argentina. The reason why there is so little *p,p'*-DDT in aquatic biota in areas where there is active application of this compound is not known. This could be due to the tropical nature of the area and that initial application is done as indoor residual spray (IRS) (van Dyk et al. 2010; Whitworth et al. 2014). The residues

Table 3: Comparable mean DDT concentration data from elsewhere in the world

Species	Locality	Situation	Organ	Year	Total DDT (ng/g ww)	Reference
Various	Rift Valley Lakes, Kenya	Farming area using DDT	Whole animal?	1970-72	74-170*	Lincer et al. 1981
Rana pipiens	Lake Erie, USA	Experimental application to a wetland	Whole animal	1963	2-550	Meeks et al. 1968
Rana catesbeiana	Lake Erie, USA	Experimental application to a wetland	Fat body	1963	470-1 300	Meeks et al. 1968
Bufo gutturalis	Zimbabwe	Farming area using DDT	Whole animal	<1987	30-3900	Table in Lambert, 2001
Ptychadena anchietae	Zimbabwe	Farming area using DDT	Whole animal	<1989	1 300-1 500	Table in Lambert, 2001
Rana clamitans	Ontario, Canada	Farming area historically using DDT	Whole animal	<1997	580-45 000	Russel et al. 1997
Rana clamitans	Ontario, Canada	Farming area historically using DDT	Fat body	1993	50-3 320	Harris et al. 1998
Rana clamitans	Michigan, USA	Forest and farmland	Whole animal	1998	1.24	Gillilland et al. 2001
Rana ridibunda	Greece	Farming area historically using DDT	Whole animal	1994	ND-30	Loumbourdis, 1997
Rana muscosa	California, USA	Mountainous region, no DDT use	Whole animal	1997	17-46	Fellers et al. 2004
Rana perezi	Ebro, Spain	Coastal wetland, legacy DDT use	Whole larvae	<2004	200 -1613*	Pastor et al. 2004
Rana perezi	Ebro, Spain	Coastal wetland, legacy DDT use	Whole animal	<2004	135 – 1315*	Pastor et al. 2004
Rhinella marina	Mexico	Industrial area	Fat body	2006	57-600	Gonzalez-Mille et al. 2013
Xenopus spp.	Limpopo South Africa	DDT-sprayed area	Fat body	2009	41-391	This study

* dry mass

from malaria control probably travel a longer route before reaching the aquatic environment than agriculturally applied DDT and therefore more *p,p*'-DDT will break down to *p,p*'-DDE.

Compared with DDT concentrations in fat bodies of frogs from elsewhere (Table 3), the present DDT concentrations were in the lower range. However, the species are different (none were *Xenopus*) and comparable lipid proportions are not available. One should also take care when interpreting Table 3, as the species, environment, dates, matrixes, and ecosystems differ widely. Overall, we conclude that *Xenopus* accumulate DDT from their environments, but that the concentrations in parts of the Limpopo province where we sampled were in the lower ranges compared to data from elsewhere. However, a more detailed aquatic assessment of the different trophic components of aquatic ecosystems will shed more light on the dynamics of DDT and other pollutants (Pastor et al. 2004).

Morphometric parameters offer a means of identifying ED effects in frogs since the process of metamorphosis and development is endocrine-mediated (Bögi et al. 2002; Jagnytsch et al. 2006). However, since the conditions of the different ponds differed and would affect growth (Harris et al. 1998), the differences in either sizes or masses (Table 2) cannot solely be attributed to DDTs. Endocrine disruption associated with DDTs has however, been detected in fish from the Luvuvhu River: intersex in male Mozambique Tilapia *Oreochromus mossambicus* was present throughout the Luvuvhu system (Barnhoorn et al. 2010).

One of the reported ED effects in laboratory exposure studies on amphibians, more specifically *X. laevis*, is masculinisation or feminisation, depending on the substance the animals are exposed to (Kloas and Lutz 2006; Cevasco et al. 2008). Kelce et al. (1995) found *p,p*'-DDE to be a potent androgen receptor antagonist. Even though the *p,p*'-DDE isomer concentrations in the males from the DDT-sprayed area was higher than the non-sprayed area (Fig. 2), no indication of feminisation (testicular oocytes) was found. The present study found a significantly narrower right testis in *X. laevis* from the DDT-sprayed area compared to the left testis (means 29 mu vs. 33 mu; Table 2). The mean length of the testes did not differ significantly between groups. Literature on asymmetry in frogs is scarce. A shorter testis length in male *X. laevis* exposed to ethinylestradiol and an increased length when exposed to methyldihydrotestosterone was found by Cevasco et al. (2008). Exposure to methoxychlor, an estrogenic chemical, resulted in reduced size and misshapen right testes of *X. tropicalis* (Fort et al. 2004). Reviews that considered abnormal testicular morphology rarely referred to asymmetry (Lutz et al. 2008; Mann et al. 2009; Rohr and McCoy 2010; Solomon et al. 2008; van den Kraak et al. 2014), but do refer to discontinuous gonads (or segmental aplasia).

We do not know the mechanism that may cause the observed asymmetry between left and right testes in the frogs. Symmetrical development of testis morphology may be mediated by endocrine systems, but we could not find direct evidence for this in the literature. Internal somatic development (especially of the circulatory system) during embryogenesis in the frog is not symmetrical (Nieuwkoop and Faber 1967), but there is eventual symmetry of organs such as kidneys and testes. Fluctuating asymmetry is the deviation from the norm between the two sides of a bilaterally symmetrical organism (Møller and Swaddle 1997) and may be caused by environmental stress during development. Fluctuating asymmetry is also an indication of the quality of the individual. Persistent organic pollutants have been associated with increased asymmetry in wing feathers of gulls (Bustnes et al. 2007). Fluctuating asymmetry in wing length, tarsus length and head length of cormorants were associated with endocrine variables (thyroid hormones, retinol and tocopherol) and persistent organic pollutants (Jenssen et al. 2010). The asymmetry in the

testes of the frogs from the DDT-spayed area, as well as a single case of an extra discontinuous left testis is therefore suggestive, but not conclusive, of endocrine disruption, possibly from DDT. More studies therefore, need to be conducted.

As a consequence of chronic long-term exposure, frogs may develop 'resistance' to DDT as suggested by Boyd et al. (1963). In Mississippi, Boyd et al. (1963) found that frogs from areas where DDT has been used for crop protection had lower mortalities when exposed to DDT than frogs from reference areas. It is not known whether this is also the case in the present situation where DDT has been applied for malaria control since the late 1940's. Exposure to DDT is likely to affect survival and fitness of frogs in various ways. DDT caused behavioural changes in *Rana temporaria* and *Bufo bufo* tadpoles in a laboratory study (Cooke 1970). In a laboratory study, Cooke (1973) found that chronic exposure to DDT may have caused kinks in the tails of *R. temporaria* tadpoles and induced hyperactivity (characterised as "frantic"). Deformities and changes in behaviour like this may expose the tadpoles to greater predation (Cooke 1973). Based on museum specimens from the USA, intersex in cricket frogs *Acris crepitans* was the highest between 1946-1959 during the greatest manufacture of DDT and PCB, and declined afterwards (Reeder et al. 2005). More effects are discussed by Carey and Bryant (1995), Lutz and Kloas (1999), and Kloas et al. (1999).

5. Conclusion

As expected, DDTs were present in quantifiable concentrations in frogs from a DDT-sprayed area, but also from non-sprayed areas. We postulate a variety of sources including DDT from malaria control and long-range transport. The presence of quantifiable DDT-derived compounds may explain its bio-accumulation in higher trophic animals such as the Grey Heron. Endocrine disruption due to DDT may have affected testicular morphology, but no testicular oocytes were found. Although only a few frogs were found after many hours of trapping, our findings raises concern about frog numbers and general health of frogs in the study area. Behavioural effects of DDT in frogs, although not studied here, may also influence predation pressure. Taken together with associations of DDT with other impacts such as intersex in male Mozambique Tilapia (Barnhoorn et al. 2010), urogenital malformations in baby boys (Bornman et al. 2010), eggshell thinning in birds from this area (Bouwman et al. 2013; Steyn et al. 2015), and the highest ever recorded concentrations of DDT in fish from South Africa, downstream of the same area inside the Kruger National Park (Gerber et al. 2016), our preliminary findings confirm the caution expressed by Ellis et al. (1944) more than 80 years ago, and add urgency to the need to reduce and eventually move away from DDT in malaria control, safely and sustainably.

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