# DEATH FROM PESTICIDES REVIEWED AMONG NON-TARGET AMPHIBIANS IN SUB-SAHARAN AFRICA

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ABSTRACT.— Pesticides are used in sub-Saharan Africa to control household, agricultural and disease vector pests. Within the context of biodiversity conservation, these complex man-made chemicals can threaten populations of non-target amphibian species in their natural habitats. Most deaths from pesticides recorded among frogs and toads in sub-Saharan Africa were due to insecticides used to control tsetse flies (*Glossina* spp.), vectors of trypanosomiasis or sleeping sickness in cattle. Organochlorine (OC) insecticides such as DDT and dieldrin, used at respectively 180 and 800 g ha$^{-1}$, caused deaths among adult *Bufo* sp., *Xenopus* sp. and *Ptychadena oxyrhynchus* in Nigeria, while endosulfan used at 200 g ha$^{-1}$ resulted in dead tadpoles in Burkina Faso, and adult *Ptychadena maccarthyensis* and *P. francisci* in Niger. Tadpoles were also killed by a toxaphene cattle-dip spill entering a river in South Africa, and by the organophosphate (OP) insecticide, iodofenphos, and a substituted di-anyl-alkane under test, during use in Ivory Coast against the aquatic larvae of black flies (*Simulium* sp.), vectors of onchocerciasis or river blindness in humans. Death among tadpoles from tsetse fly control was also caused by the pyrethroids, cyfluthrin (40 g ha$^{-1}$) in Cameroon, and deltamethrin (12.5 g ha$^{-1}$) and permethrin (11.5 g ha$^{-1}$) in Burkina Faso. A very abundant frog species *Tomopterna cryptotis*, was found, as a bioindicator of non-contamination, at the bottom of hand-dug, dry river-bed wells. To test sensitivity, two small samples were placed consecutively in contact with wetted highly contaminated soil containing an OC/OP mixture (up to 3728 ppm) from spillage of a destroyed pesticide store near Hargeisa in Somalia's North-West Zone. The frogs perished within 40-65 min, and whole body total-insecticide residue load (geometric mean at 30.9 ppm wet weight) was elevated 168 times above the baseline control value (0.2 ppm) — the OC, ß-HCH, with levels 15-times higher, was taken in more readily than dieldrin. As elsewhere in the world, amphibians were thus found to be sensitive to pesticides in countries of sub-Saharan Africa, where, commensurate with development, usage and problems associated with storage are generally on the increase.

THE effects of pesticides and other chemical contaminants on amphibians during different stages of their life cycle were reviewed some years ago by Power et al. (1989). The amphibian life cycle is biphasic, and generally involves both aquatic and terrestrial stages. Pesticides during the aquatic stage can thus affect eggs, laid as static masses or in strings embedded in gelatine, or larvae, which, as mobile tadpoles, respire by gulping water and forcing it over delicate gill membranes, with some gaseous exchange also achieved through the skin. Metamorphosis then takes place, and pesticides can affect adults during the terrestrial stage when absorbed through the skin, ingested with contaminated prey and inhaled during lung breathing. Adult anurans consume both vertebrate and invertebrate prey, while urodeles - occurring primarily in temperate zones - have a similar diet, but tend more to inhabit moist locations under logs, rocks and leaf litter, returning to the water in spring to breed.

Pesticides may be acutely or chronically toxic to amphibians, usually affecting the nervous system, and, at low levels of contamination, may be absorbed, with residues becoming concentrated in different parts of the body. Pesticides additionally cause endocrine disruption (Hayes, 2000), and may give rise to growth abnormalities and deformities in amphibians (Ouellet, 2000). With such teratogenic effects, in addition to

| Chemical | Dose (g ha$^{-1}$) | Control purpose | Observation (and country) | Citation |
|---|---|---|---|---|
| ORGANOCHLORINES | | | | |
| DDT | 180 | Tsetse flies (Nigeria) | Adult anurans killed | Koeman et al. (1978) |
| Dieldrin | 800 | Tsetse flies (Nigeria) | Toads (*Bufo* sp.) killed | Koeman et al. (1978) |
| | 900 | Tsetse flies (Nigeria) | Clawed frogs (*Xenopus* sp.) killed | Koeman et al. (1978) |
| | 1800 | Tsetse flies (Nigeria) | Frogs (*Ptychadena oxyrhynchus*) killed | Koeman et al. (1971) |
| | 3000 | Tsetse flies (Ivory Coast) | Anuran killed | Müller (1989) |
| Endosulfan | 100 (x2) | Tsetse flies (Burkina Faso) | Tadpoles killed | Everts et al. (1978) |
| | 200 | Tsetse flies (Nigeria) | Anurans killed | Koeman et al. (1978) |
| | 900 | Tsetse flies (Niger) | Frogs (*Ptychadena maccarthyensis* and *P. francisci*) killed | Dortland et al. (1977) |
| | - | Tsetse flies (general) | Amphibians killed | Müller (1983) |
| Toxaphene | - | Cattle-dip spill (South Africa) | Tadpoles killed | Brooks & Gardner (1980) |
| ORGANOPHOSPHATES | | | | |
| Iodofenphos | - | *Simulium* larvae (Ivory Coast) | Tadpoles killed | Dejoux (1978) |
| ORGANOCHLORINE/ORGANOPHOSPHATE | | | | |
| Dieldrin and products, BHC[1] isomers and heptachlor malathion, fenitrothion and diazinon | Spillage; soil contaminated 0.07-3728 ppm (1.0-5180 mg l$^{-1}$) | Locusts - destroyed store (Somaliland), May 1988 with obsolete pesticides | Frogs (*Tomopterna cryptotis*) placed onto wetted soil [0.5 l water: 1 l soil (36% dieldrin and products, 32% heptachlor, 32% ß-HCH isomers and <1% malathion)] contaminated at 3728 ppm were all dead or moribund after 40-65 min | Lambert (1997a) |
| PYRETHROIDS | | | | |
| Cyfluthrin | >40 | Tsetse flies (Cameroon) | Tadpoles killed | Müller (1989) |
| Deltamethrin | 12.5 | Tsetse flies (Burkina Faso) | Tadpoles killed | Everts et al. (1978) |
| Permethrin | 2.5+3(x3) | Tsetse flies (Burkina Faso) | Tadpoles killed | Everts et al. (1978) |
| SUBSTITUTED DI-ANYL-ALKANE | | | | |
| GH74R[2] (OMS 1358) | 0.2 ppm in water for 10 min | *Simulium* larvae (Ivory Coast) | Amphibians killed | Troubat & Lardeux (1982) |

[1]Beta-hexachlorocyclobenzene
[2]1, 1-bis-(para-ethoxyphenyl) 2-nitro propane

**Table 1**. Records of death from pesticides among non-target amphibians in sub-Saharan Africa.

hatching success, mortality and behavioural defects, tadpoles of the British Common Frog *Rana temporaria* have been used as a bioassay, when placed in cages immersed in water to assess the toxicity to amphibians of pesticide run-off and sediment-borne contamination (Cooke, 1977, 1981). The very sensitivity of amphibians to pesticides and other chemicals renders them useful bioindicators of the levels of contamination in the environment, and their presence without growth abnormalities is generally indicative of pristine conditions. With their link between invertebrate prey and predators higher-up the food chain that in turn ingest them as a trophic resource, amphibians' residue loads can also be biomarkers of the levels of pesticides entering the environment generally via faunal food chains (Lambert, 2001).

Effects of pesticides on amphibians have recently been reviewed by Cowman & Mazanti (2000) and Sparling (2000), while measurement of contamination exposure and its effects in amphibians have been reviewed by Bishop & Martinovic (2000). As part of risk assessment, the threat posed to amphibian populations by chemicals generally have been reviewed by Birge et al. (2000). From these reviews, it was noted that few studies were based on the effects on non-target amphibians of pesticides used for disease vector control and agriculture in tropical developing countries. Although both amphibian and reptile species richness is highest in the tropics (e.g. Mittermeier et al., 1992), most previous studies have been conducted in temperate zones (Devillers & Exbrayat, 1992).

The scanty information available on the effects of pesticides on amphibians in sub-Saharan Africa, based on records in the literature, and observations made during 1993 by Lambert (1997b) in Somalia's North-West Zone (Republic of Somaliland), have already been reviewed by Lambert (1997a). The few reports mentioning death from pesticides among non-target amphibians in sub-Saharan Africa are re-considered here specifically in order to draw attention, within the context of risk assessment, to the potential hazard presented by pesticides to amphibian populations in the region, and in tropical and sub-tropical zones elsewhere.

A range of pesticides causing death among amphibians in different countries of sub-Saharan Africa include organochlorine (OC), organophosphate (OP) and pyrethroid insecticides, used primarily for control of tsetse flies, *Glossina* sp., vectors of trypanosomiasis or sleeping sickness in cattle (Table 1). The majority of observations of death recorded in this work were based simply on the number of animals found dead during ground inspections following insecticide treatment against tsetse flies, or from chemical spills that may involve certain specific sampling and experimental procedures (e.g. Lambert, 1997b). For the last, it was important to show that soil highly contaminated from spillage chemicals from a destroyed store was indeed toxic and presented a threat to common and abundant species in the vicinity, especially a locally occurring amphibian species. The very presence of this species, thus demonstrated to be sensitive and without growth abnormalities, at the bottom of dry river-bed hand-dug wells served by ground water and used by local people, was an indication that ground water below the spillage area remained uncontaminated.

**Organochlorines**

Quite early on, OC pesticides were recorded to have caused havoc to wildlife (e.g. Carson, 1962).

*DDT* - Dichlorodiphenyltrichloroethane (DDT) is an environmentally persistent pesticide that in recent years has been banned in many countries. The mechanism for toxicity of DDT, although mainly due to a physiological response, can in part be due to behavioural aberrations, and disruption of glandular development may cause morphological abnormalities. Death among adult amphibians in sub-Saharan Africa was recorded from DDT-spraying against tsetse flies at a concentration as low as 180 g $ha^{-1}$ (Table 1).

*Dieldrin* - Dieldrin is known to be highly toxic to homeothermic birds and mammals (IPCS, 1989). The primary site of action of aldrin-transdiol (the active metabolite of dieldrin) is somewhere in the central nervous system, with an increase of polysynaptic reflex activity and reduction of orthodromic postsynaptic inhibition, so that excitatory effects were followed by reduced spinal excitability. Many deaths among reptiles resulted from dieldrin-spraying against tsetse flies at the relatively low dose of 200 g $ha^{-1}$ in Zambia (Wilson, 1972), and death was also recorded among anurans (frogs and toads) (Table 1).

*Endosulfan* - Endosulfan is not known to be persistent (Douthwaite, 1986), and this is an advantage it has over OC insecticides such as DDT. There is a reduced swimming capacity and lack of physical stamina, and a thinning and transparency of the gills were observed symptoms of poisoning. Applications against Tsetse Flies (lowest dose at 200 g $ha^{-1}$) caused death among tadpoles and adults (Table 1).

*Toxaphene* - Toxaphene causes behavioural aberrations (mainly hyperirritability and prolonged stupor) and growth retardation. A toxaphene cattle-dip spill entering a river caused death of tadpoles in South Africa (Table 1).

*Beta-hexachlorocyclobenzene* (technical BHC) - ß-HCH, in combination with dieldrin from heavy pesticide spillage, was especially toxic to frogs (Table 1). ß-HCH residue levels in a toxicity test sample (n = 5) of resultant dead frogs had a geometric mean of 27.09 (range 2.58-322.95) ppm whole body wet weight which was elevated 301.0 times above the 0.09 (range 0-0.43) ppm in controls (n = 7) (Lambert, 1997a).

**Organophosphates**

Organophosphate insecticides cause inhibition of acetylcholinesterase enzymes, essential for nerve impulse conduction and transmission, which may lead to aberrant behaviour placing the organism at a selective disadvantage and resulting in increased predation.

*Iodofenphos* - Used against larvae of black flies, *Simulium* sp., the vector of onchocerciasis or river blindness in humans, iodofenphos was recorded to cause death among tadpoles in a river in West Africa (Table 1).

*Spillage chemicals (OCS and OPS)* - Due to spillage from a destroyed pesticide store near Hargeisa, Somaliland, soil covering an area of 3700 $m^2$ was contaminated at up to 3728 ppm total insecticides (Lambert, 1997b). Death resulted when adult frogs of abundant *Tomopterna cryptotis* were placed experimentally in contact with the wetted, highly contaminated soil containing OCs (99.6%): dieldrin and products (36.0%), ß-HCH isomers (31.5%) and heptachlor (32.1%), and the OPs (0.4%): malathion, fenitrothion and diazinon (Table 1). No frogs in a first experiment (n = 12) remained alive after 40 min (50 and 80% dead or moribund after respectively 10 and 20 min), and in a repeat experiment (n = 11), all but the two largest individuals — which respectively survived 15 and 20 min longer — were dead after 45 min. Frogs kept alive on uncontaminated mud from hand-dug wells (n = 92 and 81 in the first and second experiments, respectively), from which samples were removed for testing, acted as controls. Total insecticide residue levels in a toxicity test sample of resultant dead frogs (n = 5) had a geometric mean of 30.88 (range 3.69-326.66) ppm whole body wet weight, compared to 0.18 (range 0.01-0.48) ppm in control animals (n = 7), and level at mortality was therefore elevated 167.8 times (Lambert, 1997b). ß-HCH was apparently taken in more readily than dieldrin, and the geometric mean was 15.0 times higher than dieldrin (ß-HCH was only 3.6 times higher than dieldrin in control frogs). With lower residue level at death, dieldrin was probably of higher toxicity to frogs than ß-HCH.

**Pyrethroids**

Pyrethroid insecticides are recognised as typical neurotoxicants (Vijverberg et al., 1982), acting on the peripheral nervous system, and inducing repetitive activity in sensory nerve fibres, sense organs and the distal portion of the motor fibres resulting in repetition in the motor end-plate. Pyrethroids have high toxicity to insects and a short half life, so that they are non-persistent in the environment. Deaths in tadpoles resulted after treatment with cyfluthrin, deltamethrin and permethrin against tsetse flies (Table 1).

## DISCUSSION

Most of the deaths from pesticides among amphibians in sub-Saharan Africa involved OC insecticides, used for control of tsetse flies, the insect vector of trypanosomiasis or sleeping sickness in cattle. DDT treatment at 180 g $ha^{-1}$, dieldrin at 800 g $ha^{-1}$ (lowest recorded for amphibians) and endosulfan at 200 g $ha^{-1}$ all caused death among adult amphibians.

Amphibian deaths resulting from DDT applications in the field depend on dose, application method, weather at time of application, depth of pond and canopy cover (Power et al., 1989). DDT treatment against mosquito larvae in ponds at 110 g $ha^{-1}$ caused no mortality to tadpoles of the North American Bull Frog *Rana catesbiana* of varying stages and sizes, although 80%

mortality within 48 hr was the result of 1000 g ha$^{-1}$ (Mulla, 1963). Routine spraying of coastal plain ponds against mosquito larvae in Georgia at 110 and 450 g ha$^{-1}$ also caused some deaths among tadpoles and adults of unspecified frog species (Tarzwell, 1950), suggesting that 180 g ha$^{-1}$ used against tsetse flies in Nigeria (Koeman et al., 1978) must be about the lowest dose that causes death in anurans. Dieldrin doses of 110 and 560 g ha$^{-1}$ applied to a pond in California were effective in controlling tadpoles of *R. catesbiana* (Mulla, 1962), and these values are still lower than the lowest at which death was recorded among adult anurans of 800 g ha$^{-1}$ when used against tsetse flies in Nigeria (Koeman et al., 1978). No amphibian deaths from applications of endosulfan are recorded by Power et al. (1989). However, Sanders (1970) estimated median tolerance limits (TL50) - the concentration of endosulfan at which half of the test animals (4 to 5-week-old tadpoles) survived during a specified exposure period (24 hr) - in two North American anurans, and found that in respectively Fowler's Toad *Bufo woodhousei fowleri* and the Chorus Frog *Pseudacris triseriata*, TL50 was at 0.6 (0.3-1.2) ppm and 1.7 (0.5-3.2) ppm.

Tadpoles were also killed by the OP, iodofenphos, used against the aquatic larvae of black fly *Simulium* sp. (Dejoux, 1978). Organophosphate insecticides are toxic to most organisms, causing erratic behaviour that may place the organism at risk of increased predation (Power et al., 1989).

Tadpoles are highly sensitive to pyrethroids, which to a degree have taken over from DDT for control of malaria mosquitoes in sub-Saharan Africa, and deaths have been caused from use of cyfluthrin, deltamethrin and permethrin against tsetse flies. Pyrethroids are a group of insecticides developed from a natural substance (pyrethrum), and are of special interest because of their short half life and their high toxicity to insect pests. However, frogs were found to be highly sensitive to 11 different pyrethroids, and the cis-isomers of deltamethrin, (1R)-permethrin and (1R, aS)-cypermethrin were the most toxic to adults of the North American Leopard Frog *Rana pipiens*; tadpoles of *R. catesbeiana* were especially sensitive to the last (Cole & Casida, 1983).

Pesticide use commensurate with development is still on the increase in sub-Saharan Africa (Sridhar, 1989), and may double within the next decade or so. Recent arguments have, however, been put forward for the continued use of DDT in South Africa (Bouwman, 2000). Pesticides contribute substantially to the alleviation of poverty, especially among rural communities, and have an important role to play in both crop pest control, which increases the productivity of arable land, and in the control of intermediary host vectors of such diseases as trypanosomiasis in cattle, and malaria, onchocerciasis and schistosomiasis among local people. However, this greater use of pesticides is associated with increasing environmental risk. A threat is also thus presented to amphibians, being sensitive to a wide range of pesticides, in both their aquatic oval and larval, and terrestrial adult stages, and such chemicals have contributed to the worldwide decline of amphibian populations (e.g. Wake, 1991; Corn, 2000), and probably to batrachian diversity in general.

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