ENVIRONMENTAL POLLUTANTS AND DISEASES OF SEXUAL DEVELOPMENT IN HUMANS AND WILDLIFE IN SOUTH AFRICA: HARBINGERS OF IMPACT ON OVERALL HEALTH?

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Abridged title

EDC and DSD IN HUMANS AND WILDLIFE OF SOUTH AFRICA

Abstract

This study deals with disorders of sexual development in humans, wildlife and animals in an urban nature reserve (RNR) and a currently DDT-sprayed malarial area. High levels of oestrogenic chemical residues in water, sediment and tissue; skewed sex ratios; reduced biodiversity; gonadal malformations in sharptooth catfish and freshwater snails; intersex in catfish; and impaired spermatogenesis in catfish and striped mouse are of serious concern in the RNR. Persistent eggshell thinning in African darter eggs, intersex in male Mozambican tilapia, follicular atresia in females and impaired spermatogenesis in males following laboratory exposure of parent fish to environmentally relevant DDT and DDE concentrations, and abnormalities in freshwater snails were found in the DDT-sprayed area. Human studies related to DDT exposure indicated impaired semen quality, a weak association with sperm chromatin defects and higher risks for external urogenital birth defects in those who were born to mothers whose houses were sprayed and those who were homemakers (stay at home mother) instead of being employed. These findings indicate that diseases of sexual development occurred in both human and wildlife populations exposed to environmental endocrine disruptor chemicals in South Africa. The chemical mixtures, possibly related to disorders of sexual differentiation (DSD),

were very different between the two. However, DSD occurred concurrently in the malarial area, possibly indicating that humans and wildlife shared exposures. Moreover, it emphasizes the importance of suspecting disease in the other when disease is found in either human or wildlife populations.

INTRODUCTION/BACKGROUND

The word "Africa" evokes images of a rich diversity of iconic wildlife. Although the faunal diversity of the continent is largely intact, many species suffer range contractions and local extinctions. However, Africa's islands with their unique biota have seen extinctions and many extant species cling on for survival (Wilson, 1999). Africa is the second largest continent and the cradle of humankind, where humans have evolved side by side with wildlife for more than six million years. Africa straddles the equator and has many climatic zones that support rain forests, savannah and deserts. It is home to about a billion people who are mainly located along the coasts and inland waters. The distribution pattern of water is patchy, leaving large regions thinly populated. The association of humans with water also means that wildlife in need of water shares the stress of decreasing water quantity and quality (Osibanjo et al., 2002). One of the major challenges is how to balance the conflicting needs of humans and wildlife amidst the persistent poverty of its rural people, rapid human population growth, and the resulting misuse and degradation of the land. These and many other factors jeopardize the future of wildlife in Africa, and a rich natural resource may be lost before its true value is fully comprehended (Bhashare et al., 2001).

Endocrine disrupting chemicals (EDCs) are synthetic chemicals that interfere with hormone action, and create adverse health effects in humans and wildlife. EDCs include industrial chemicals, pharmaceuticals, personal care products, metals, and pesticides (Toppari et al., 1996). Already in 1994 Colborn warned that the environmental load of EDCs has reached such critical levels that humans and wildlife are at risk. Sexual differentiation during foetal development is a dynamic and sequential process including the establishment of chromosomal sex at fertilization, which determines development of the undifferentiated gonads into testes or ovaries, and subsequent sexual differentiation resulting from endocrine functions associated with the type of gonad present (Jost et al, 1973). Interference with this highly ordered process at any step could result in a disorder of sexual differentiation (DSD) (Snodgrass, 2012). The increased rate of cryptorchidism, hypospadias, testicular cancer, and male infertility (testicular dysgenesis syndrome or TDS) in humans may be the result of prenatal exposure to endocrine disruptors (Skakkebaek et al. 2001).

The Republic of South Africa (SA), at the southern tip of the African continent, is ranked sixth on biodiversity in the world. Because of the large diversity in combinations of geography and climate, SA hosts a diversity of habitats that is arguably unsurpassed by any other comparable sized region (Harrison et al., 1997). Nature conservation is a national priority through national, provincial, and private parks, World Heritage Sites, peace parks, and conservancies. Increasing population and industrialization pollutes water that also affects wildlife. Since most of the African human population is also closely dependent on their immediate surroundings for subsistence, polluted water would also be a threat to humans; the nexus between humans, wildlife, and water is a major feature of the pollution situations described below. A disease/disorder in the one may be an early signal/precursor event of a threatening disease/disorder in the other. This paper deals with disorders of sexual development in humans, wildlife, and animals and discusses the human-animal interlinkage serving as reciprocal harbingers of impacts.

DISEASES OF SEXUAL DIFFERENTIATION IN WILDLIFE FROM AN URBAN NATURE RESERVE

The Rietvlei Nature Reserve (RNR), close to the metropolitan areas of Johannesburg, Ekurhuleni, and Tshwane, is one of the world's largest urban nature reserves. The Riet Spruit flowing into the RNR receives effluent from sewage treatment plant, industries, and informal settlements. In view of the unique combination of anthropogenic, industrial, and agricultural activities close to an urban conservation area, the South African Water Research Commission (WRC) identified RNR as a National Priority Area for monitoring EDCs. The study aimed to determine whether sufficiently high levels of EDCs exist in the RNR environment to exert adverse health effects on aquatic or terrestrial animals and humans. Water and sediment samples from the Marais Dam (MD) and Rietvlei Dam (RVD) collected every two months for two years were analyzed for target chemicals and biological assessment of water for estrogenicity (recombinant cell bioassay, Yeast-based recombinant estrogen receptor- assay YES) and the ER-Calux® reporter gene assay expressing endogenous estrogen receptor (ER)α- and β-. The rat hepatoma cell line H4IIE a reporter gene bioassay to assess dioxin and dioxin-like PCBs, was used on sediment samples. Sharptooth catfish, freshwater snails, eland, and striped mouse were evaluated as possible aquatic and terrestrial biosentinels for EDC exposure (Bornman et al., 2007).

Water and sediment in RNR

The chemical composition of the water was a mixture of agrochemical pesticides and industrial pollutants at levels of concern for human consumption. The list included cyclohexane, lindane, endrin, heptachlor epoxide, methoxychlor, DDT and metabolites, octylphenol (OcP), p-nonylphenol (p-NP), dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP). All the samples contained detectable DEP and DEHP residues. Lindane was found in 31.4% and p,p'-DDT in 25,7% samples. Sediment did not have any detectable dioxin or dioxin-like PCBs, but had p,p'-DDD in 7/35 (20%) samples, p-NP in 40% samples and also phthalate ester residues (Bornman et al., 2007). High concentrations of phthalates are frequently reported from less developed countries, due to lack of legislation and/or facilities to treat industrial effluents (Fatoki and Ogunfowokan, 1993). Endocrine disruptive metals were present, with cadmium above the chronic effect value and lead above the acute effect value (DWAF, 1996).

These chemicals in drinking water may individually or as mixture pose a risk to humans and wildlife. Using untreated water from RNR for vegetable watering (as would be the case with subsistence gardening in the informal settlement area upstream) pose human cancer risks between 2.3 and 2.7 per 1000. Hazard indices indicated that the water had 25-35 times the chemical concentrations considered safe for a lifetime exposure. The chemicals of concern were lindane, DDT and metabolites, DBP, and DEHP. Even with an 80% reduction scenario, based on the percentage removal associated with the Rietvlei wastewater treatment plant (Snyder et al., 2003), the water still posed a risk to human health. Collectively, the best way of addressing chemical contaminants in RNR would be measures to control the effluent from different sources entering Riet Spruit.

The YES assay demonstrated estrogenicity in 35% of water samples, but cytotoxicity occurred in 69% samples, indicating that estrogenic activity could be masked at these concentrations as the estrogenic response may lie in the toxic range. The ten samples tested in the Netherlands using the ER-Calux® all had estrogenicity and in 80% the estrogenicity was > 1ng/L (Bornman et al. 2007). This value is the critical level for producing a risk to reproductive damage in exposed fish (Matthiessen et al., 2006).

Biosentinel species in RNR

Sharptooth catfish (*Clarias gariepinus*) comprises an ecologically diverse component of most river faunas of much of southern Africa and is a relatively hardy species (Skelton, 1993). Barnhoorn et al. (2004) reported the first cases from Africa of testicular oocytes (intersex) in male catfish from the RNR; there was also a skewed male: female ratio towards females. Intersex is the development of testicular oocytes in a male gonad (Gray and Metcalf, 1997; Metcalfe et al., 2001) resulting from embryonic exposure to ED chemicals (Jobling and Sumpter, 1993) like alkylphenols OcP and p-NP and DDT and metabolites (Jobling et al., 1998). Bornman et al. (2007) reported an overall incidence of 28.9% intersex in male catfish (n=80). Fish fat from both dams contained detectable residues of lindane, aldrin, o,p'-DDT, p,p'-DDT, p,p'-DDD, o,p'-DDE, p,p'-DDE, PCB153, OcP, and p-NP.

Freshwater molluscs represent a significant component of Africa's biodiversity (460 species; Appleton, 1996) and an important part of the aquatic ecology. Possible endocrine disruption of male reproductive organs of the freshwater mollusc *Bulinus tropicus* was investigated. *B. tropicus* from RNR and from a reference wetland were narcotised, preserved, and the penis complex dissected. The lengths of the penis sheath and preputium was microscopically measured; the penis sheath:preputium length ratio (PSPLR) calculated and compared to historical preparations from the National Snail Collection (NSC at the North-West University). Six snails from RNR had no penis, a very rare phenomenon for this species. There was no difference between the NSC and reference PSPLR means ($p > 0.05$), but significant differences between NSC and RVD ($p < 0.001$), and reference RVD PSPLR means ($p<0.05$). Therefore, at RVD the penis sheath showed reduction relative to the preputium, possibly associated with endocrine disruption at RVD (Bornman et al., 2007). Preliminary laboratory exposures of *B. tropicus* hatchlings to bisphenol A, p-NP, and o,p'-DDT at 0, 0.1, 5 and 100 µg/l affected the PSPLR. Agenesis of the penis was seen in o,p'-DDT exposed individuals, but further laboratory testing is needed to confirm cause-effect relationships. Given the relative ease of sampling and low-cost equipment required, PSPLR maybe a useful quantitative biomarker of ED exposure in freshwater snails.

During culling operations in the RNR, white focal, gritty areas of calcifications were observed in the testes of an eland antelope (*Tragelaphus oryx*). Other male eland were subsequently examined and all 17 animals had similar lesions. On histology, multiple intratubular dystrophic calcifications, focal areas of sperm stasis, and interstitial chronic cell infiltrates with fibrosis occurred. Impaired spermatogenesis, a few atypical germ cells resembling carcinoma *in situ* (CIS), vacuolization of Sertoli cells and sloughing of the seminiferous epithelium were present, similar to human TDS (Bornman et al., 2010).

The association of testicular calcifications together with a variety of testicular lesions was found in beef bulls with small testes (Veeramachaneni et al., 1986) and subsequently in Sitka black-tailed deer from Kodiak Island in Alaska (Veeramachaneni et al., 2006). These deer had both testicular and antler dysgenesis and the authors suggested that exposure of pregnant females to an estrogenic environmental agent(s) seems likely. Subsequently, testicular calcifications were also found in eland from another nature reserve and in both instances, the fatty tissues contained significant levels of bio-accumulated estrogenic environmental contaminants (Bornman et al., 2010). The adenomatous changes of the eland rete testis were similar to diethylstilbestrol-induced rete adenocarcinoma in laboratory animals (Newbold et al., 1985; Newbold, 2000) and was, therefore, also suggestive of possible chronic estrogenic exposure. Striped Mouse (Rhabdomyspumilio) collected in the RNR also had impaired spermatogenesis, apical sloughing of the germinal epithelium, degeneration of spermatogonia, vacuolization, and seminiferous tubule shrinkage (Bornman et al., 2007). The testicular findings (excluding calcifications) are similar to rat testes following experimental exposure to *p*-NP (De Jager et al., 1999b). McClusky et al. (2007) demonstrated that p-NP insidiously modifies the spermatogenic cycle and spermatogenic process in male offspring, and enhances germ cell apoptosis.

Although no method was available to calculate the long-term risk of EDCs on the ecosystem of RNR, the findings of high chemical residue levels in water, sediment and tissue, skewed sex ratios, reduced biodiversity, gonadal malformations in sharptooth catfish and freshwater snails, intersex in catfish, histological impacts on spermatogenesis in catfish and striped mouse are matters of serious concern. It is highly unlikely that such a diversity of effects in a range of biosentinel animals could be coincidental (Bornman et al., 2007). These findings are the first indications of mammalian wildlife being affected by environmental pollution of endocrine disrupter chemicals in South Africa (Bornman et al., 2010).

WILD BIRD EGGS FROM THE VAAL RIVER

Historically, reproductive endocrine disruption and eggshell thinning in predatory birds prompted the ban on the use of DDT in North America and Europe. The subsequent reduction in body burdens in birds and an improvement in eggshell thickness, led to subsequent recovery of the affected populations (Cheek, 2006).

South Africa's bird diversity is exceptional with 928 species of which 161 are endemic or near-endemic (Maclean, 1999). It is well known that birds are susceptible to EDCs, and this threat has been detected in southern Africa. In the 1970's and 1980's, much work was done on levels and effects of pollutants in wildlife in Zimbabwe and South Africa (De Kock and Boshoff, 1987), but since then, little research has been published despite the high levels previously found. Eggs from African darter, cattle egret, reed cormorant, African sacred ibis, as well as single eggs from some other species collected from the Vaal River had detectable levels of HCB, DDTs, HCHs, chlordanes, and PCBs (Bouwman et al., 2008). The African darter eggs had the highest levels of all other compounds except for chlordane. Eggshell thinning was detected in the African darter, and was associated with most of the compounds, including DDE, chlordanes, β-HCH, and PCBs, but not with HCB, p,p'-DDT, or mirex. With an increase of 55 ng/g^{-1} ∑PCB, a 17% decrease in eggshell thickness was found, but it is likely that the eggshell thinning was a result of the combined effects of the chemicals rather than just p,p'-DDE (Blus and Henny, 1997). The African darter could also be more sensitive to the effects of, for example, p,p'-DDE (Blus and Henny, 1997). Bouwman et al. (2006) expressed concern about the reproductive health of the African darter and other birds in the region such as the iconic African fish eagle (*Haliaeetus vocifer*).

DISEASES OF SEXUAL DIFFERENTIATION IN A CURRENTLY DDT-SPRAYED AREA, LIMPOPO PROVINCE

In animals, adverse effects on the male reproductive system are associated with *in utero* DDT or DDE exposure, including reduced penis size (Guillette et al., 1999), hypospadias (Gray et al., 2001), cryptorchidism (Facemire et al., 1995; Gray et al., 2001), and abnormal development of ovarian tissue (Fry and Toone, 1981). Here, we deal with findings of DSD in wildlife and humans in an area of chronic DDT exposure. After indoor residual spraying (IRS) for malaria vector control, the sprayed DDT may reach the outdoor environment via air and dust and from possible spillages during application.

Malaria mosquitoes lay their eggs in water, thereby complicating the human, wildlife, and water scenario investigated in this paper. DDT is the environmental pollutant most often reported on (UNEP Report). Although all countries have banned the use of DDT in agriculture, it may still be used for malaria control. DDT is not only a persistent organic pollutant (POP), but also an endocrine disruptor (ED). Technical DDT, which is applied during IRS, is composed of 65–80% of the active insecticidal ingredient p,p'-DDT and 15–21% of the less insecticidal o,p'-DDT . Technical DDT has oestrogen-like properties largely due to o,p'-DDT), while p,p'-DDT is metabolized to the persistent p,p'-DDE, a potent anti-androgen (Bouwman, 2006).

DDT residues and effects

Fat tissue samples from the Mozambique tilapia *O. mossambicus* from an area where DDT is sprayed had significantly ($p < 0.0005$) higher (20-times) higher ∑DDT levels than from a reference area (Barnhoorn et al., 2009). In a subsequent paper Barnhoorn et al. (2010) reported intersex in 49% of *O. mossambicus*, indicating that DDT residues are likely involved in the development of intersex. Mlambo et al. (2009) determined the histopathological changes in the reproductive system (ovaries and testes) of *O. mossambicus* following laboratory exposure to environmentally relevant concentrations of technical DDT for 40 days. Fertilized eggs were artificially incubated. In the 5g l^{-1} exposure, post-hatch survival was significantly lower, and prevalence of larval skeletal deformities significantly higher compared to the control ($p < 0.05$). DDT caused increased oocyte atresia in the ovaries and disorganization of seminiferous lobules in the testes of adults. Moreover, DDT exposure reduced survival and increased deformities in larvae at levels that did not cause severe histopathological changes to parental gonads.

PSPLR of *B. tropicus* from DDT-sprayed areas were compared to reference snails from a non-DDT sprayed area to determine if this species can be used as a biological indicator. Statistical analysis showed significant differences in PSPLR (and therefore possible endocrine disruption) between snails from the two areas. The difference in PSPLR values was mainly due to a relatively shorter preputium from snails in the DDT-sprayed area. Although sediment samples from where the snails were collected showed detectable DDT present in most of the DDT-sprayed sediments and less so in the non-DDT sprayed sites, causality of the possible ED could not be established from this field study. This study indicated the possibility of using the PSPLR as endpoint for ED (WRC study – in prep).

Eggs collected from grey heron (*Ardea cinerea*) and cattle egret (*Bubulcus ibis*) from DDT-sprayed and non-DDT sprayed areas (WRC report, in prep) were analyzed for organochlorine compounds. Only terrestrial bird eggs could be collected in the DDT-sprayed area as no breeding colonies of water birds were found here. HCH, HCB, mirex, PCBs, and DDT were detected, with DDT at the highest levels. The grey heron had very high levels of DDT in their eggs (3.2 mg/kg ww) probably reflecting the high DDT levels found in fish (Barnhoorn et al., 2009). The DDT level found the grey heron eggs was the same as the level deemed critical for reproductive success found for brown pelicans (USDoI, 1998). DDE concentrations of 2.8 mg/kg ww have been associated with reproductive impairment of piscivore bird populations, while 1 mg/kg ww was linked to reduction of heron survival (Connell et al., 2003). Relatively lower DDT levels were found in cattle egrets, but these levels were still higher than from elsewhere in South Africa (Bouwman et al., 2008). It was considered possible that the high DDT levels in fish from the DDT-sprayed area might adversely affect reproduction of aquatic birds that prey on them (WRC report, in prep).

Human exposure and health effects in Limpopo

Since DDT is used for IRS to control malaria vectors, residents could be exposed to residues of DDT through various pathways including indoor air, dust, soil, food, and water. Van Dyk et al. (2010) measured levels of DDT in representative homesteads and possible routes of human exposure. Human serum, indoor air, floor dust, outside soil, potable water, leafy vegetables, and chicken samples (muscle, fat and liver) were analyzed for the o,p′- and p,p′-isomers of DDT, DDD and DDE. DDT was detected in all the media analyzed indicating a combination of potential dietary and non-dietary pathways of uptake. The results raise concerns regarding the potential health effects in residents living in IRS treated residences. More importantly, the levels measured in the air, vegetables, and chickens were above the WHO guideline values for human safety. In addition, any breast feeding infants will be exposed to air, indoor dust, outdoor soil, and some food, as well as high levels of DDT in breast-milk (Bouwman et al., 2006; Sereda et al., 2009), further increasing health risks to infants.

Young adult healthy men (n=311) from an endemic malarial area had very high mean lipid-adjusted blood levels (p,p’-DDT and p,p’-DDE; (90 mg/g and 215 mg/g), but body burdens were lower in those whose houses were not sprayed (Aneck-Hahn et al, 2007). The number of cytoplasmic droplets and ejaculate volume was also adversely affected. Low sperm counts were significantly positively associated with p,p’-DDE (odds ratio [OR]=1.001, P=0 .03) and impaired sperm motility significantly positively associated with p,p’-DDT (OR 1.003, P =0.006) and p,p’-DDE (OR =1.001, P=0.02). These results indicated that IRS exposure to DDT is associated with impaired seminal parameters in men. De Jager et al. (2009) measured possible sperm chromatin defects in this population and found a weak association between DDT/DDE plasma concentration and the incidence of sperm with chromatin defects. The results suggest that DDT exposure via IRS may have a negative impact on sperm chromatin integrity in young males.

Bornman et al. (2010) determined the association of external urogenital birth defects (UGBDs) in newborn boys from DDT-sprayed and non-sprayed villages in a malarial area where the mothers were living in comparable ethnic and socio-economic backgrounds. A multivariate logistic model showed that mothers living in villages sprayed with DDT between 1995 and 2003 had a 33% greater chance of having a baby with a UGBD than mothers whose homes were not sprayed (OR 1.33, 95% confidence interval 1.04–1.72). Being a homemaker (stay at home mother) instead of being employed significantly increased the risk of having a baby with a UGBD by 41% (odds ratio 1.41, 1.13–1.77). Further studies are necessary to determine possible causal relationships of IRS and DDT exposure and any possible genetic or epigenetic predispositions.

In the human studies not only reproductive health of humans and wildlife is affected, but changes in retinol-binding protein concentrations and thyroid homeostasis occurred with exposure to DDT, and these may have deleterious effects on thyroid function and vitamin A nutritional status. This is of particular concern in a population with poor vitamin A and zinc intake (Delport et al., 2011).

DISCUSSION

The above descriptions show that in scenarios where water, wildlife (including mosquitoes), and humans interact, chemicals such as PCBs, nonylphenol, and DDT have been associated with effects on reproductive and developmental parameters in humans and wildlife. Putting together the data presented above, humans and wildlife appear to be exposed to environmental EDCs in a similar manner. While human populations may be highly exposed to single chemicals such as DDT, wildlife is exposed to mixtures of chemicals that end up in their environment. Findings in wildlife cannot be ignored because at the time when effects in humans occur, it may be too late for interventions to make a difference.

These studies above represent only some of the potential human, wildlife, and water interactions with chemicals that occur in Africa. The subsistence conditions under which many people live in Africa underscores the direct relations that people have with their environment. Pollutants affect people and wildlife often via the same pathways and in the same proximity. Effects seen in wildlife may therefore indicate effects in humans and vice versa. A better understanding of the complex interactions is therefore needed to reduce or prevent exposures and thereby improve human and wildlife health.

Scenarios where chemicals interact closely with humans and wildlife in the same proximity as water, is an under-researched topic. Many other components of wildlife interact closely with humans in African settings. In a more developed-country setting, pets are often used as an indicator of human exposure. In Africa, small-mammals such as mice and bats, reptiles such as snakes and lizards, birds, and insects have co-existed with humans for six million years probably reflecting a closer relationship than elsewhere. In the context of ecotoxicology, the myriad of interactions remain to be explored, promising a rich source of data that may indicate how human and wildlife health can be promoted under co-existence.

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Conflict of interest

None

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