Developmental disorders induced by pesticide degradation products

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Publication date
2002

Citation for published version (APA):

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Fate and Risk of Chloroacetanilide Degradation Products in the Nzoia Basin, Kenya

Abstract

Alachlor, metolachlor and their respective environmentally stable aniline degradation products, 2,6-diethylaniline and 2-ethyl-6-methylaniline were analyzed in water and sediment samples from 9 sites along River Nzoia, Kenya using gas chromatography. The degradation products were detected in >90% of the sediment and water samples, while the parent compounds occurred in <14% of the water samples. Much higher concentrations of the pesticides and their degradation products occurred in the sediment than in the water (1.4 - 10800 fold), indicating an accumulation of the compounds in the sediment. The constant occurrence of the degradation products in the sediment during the study period infers a persistence of these compounds. It is hypothesized that the prevailing tropical climatic conditions favor a quick breakdown of the pesticides to their environmentally stable degradation products, thereby making the latter more important pollutants than their parent products in the study area.
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**Introduction**

Potential risks of pesticide use in the tropics may differ from those in the widely studied temperate regions. It is generally assumed that the climatic conditions in the tropics facilitate the breakdown of bioactive compounds, thus avoiding much of the side effects of pesticides. On the contrary, we hypothesize that degradation products of bioactive compounds play a prominent role under the tropical conditions and we use the River Nzoia basin in Kenya as a case study. Kenya, like most other developing countries, is at the crossroads of an environmentally sustainable agricultural practice and ostensible economically sustainable practices, characterised by a high usage of agricultural chemicals. The current rural to urban migration necessitates the use of herbicides as an easier alternative to the age-old labour intensive manual weed control. Also, the pressure of a fast growing population calls for more intensification of agriculture with an attendant increased use of agricultural chemicals, in Kenya as well as in other tropical countries (Lacher and Goldstein 1997). Pesticide use in Kenya is already one of the highest in sub-Saharan Africa with a market share of approximately US$ 40.4 million by 1992 (Partow 1995).

After farm application, pesticides and their degradation products dissipate into other environmental compartments including groundwater, surface water, and the atmosphere. Degradation of chloroacetanilide herbicides after use has been shown to result in several products with 2,6-diethylaniline and 2-ethyl-6-methylaniline being the environmentally stable end products for alachlor and metolachlor, respectively (Kimmel et al. 1986; Tessier and Clark 1995; Gonzalez-Barreiro et al. 2000). The rate and extent of degradation depends on the microbial composition of soil or sediment (Bollag et al. 1986; Liu et al. 1995; Stamper and Tuovinen 1998), temperature (Hamaker 1972; Gerstl 1991), moisture content (Gerstl 1991), hydroxyl radical ("OH) generating processes (Webster et al. 1998), and uptake and degradation by resistant plants (Feng 1991; Field and Thurman 1996; Aga and Thurman 2001). A common soil fungus, Chaetomuina globosum (Tiedje and Hagedorn 1975) and sediment inhabiting chironomid larvae (Wei and Vossbrinck 1992) have also been shown to degrade chloroacetanilides. Variability in environmental half-lives of organic pesticides at different climatic conditions is widely reported (Webster et al. 1998). It is possible that the herbicides degrade faster in the tropics due to
the higher ambient temperatures, conducive for microbial activities, metabolism and/or photolysis. The chloroacetanilide degradation products have been detected in surface and ground waters of the temperate agricultural zones (Phillips et al. 1999; Scribner et al. 2000), and due to their persistence, they are important environmental contaminants. Moreover, these chloroacetanilide stable aniline degradation products are more or equally toxic compared to their parent pesticide compounds (Osano et al. 2002b; Osano et al. 2002a) and are promutagens in the Ames test (Kimmel et al. 1986). Hence, we investigated the extent of contamination of river Nzoia with the chloroacetanilide herbicides alachlor, metolachlor and their environmentally stable aniline degradation products 2,6-diethylaniline, and 2-ethyl-6-methylaniline and reconstructed the fate of the pesticides in the basin. The sequel of heavy use of the chloroacetanilides is collated with possible ecotoxicological effects of the compounds.

The Study Area

The Nzoia River basin is approximately 1 269 600 hectares and lies entirely within the lake Victoria basin in Kenya, East Africa. It is bounded by the latitudes: 34° - 36° east, longitude: 0°03’ - 1°15’ north, and lies between 1134 - 2700 m above sea level. It encompasses three geographical regions: the highlands around Mount Elgon and the Cherangany Hills, the upper plateau, which includes Eldoret, and the lowlands. The region receives an average of 1350 mm/year of rain and is an important cereal and sugarcane-farming region of Kenya producing at least 30% of the national output of both maize and sugar. Potential major sources of pollution for the river are the agricultural chemicals, urban effluents of Eldoret (population 234000), Kitale (pop. 88100), Bungoma (pop. 32900), Webuye (pop. 45100), Kakamega (pop. 86500), Mumias (pop. 36200), industrial wastes of the Panpaper pulp mills at Webuye, textile factories in Eldoret, coffee factories scattered in the higher regions, and sugar industries mainly at Mumias, Kakamega and Bungoma districts. The polluting role played by any of these factors is unknown. Herein, we study the role played by agricultural chemicals, specifically the chloroacetanilide herbicides, as agriculture is still the predominant activity in the region (Fig 1). The total length of the river is ca. 252 km with average fall of 4 per 1000 (JICA 1987).
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Nine stations were sampled along the river and its tributaries. Station 1 is a pristine upstream site, although it is located amidst an upland agricultural land. It drains a protected natural forest reserve and the peak of Mt. Elgon. Contamination of the river at this point is unlikely. Station 2 lies within large-scale maize farms in Endebess. Station 3 drains large-scale maize and wheat farms of approximately 15,000 ha., which use herbicides almost exclusively to control weeds. The site drains the Agricultural Development Corporation farm (approx 11,000 ha.), the Kenya Seed farm and Kitale Municipality. Station 4, drains approximately 100,000 ha. watershed, all the above stations and some additional large-scale and small-scale maize farms. The large-scale maize farms probably use chemicals to control weeds. Station 5 is a detour of the river at station 4 into a swamp. Station 6, draining approximately 107,270 ha. watershed, lies 1 km downstream of the Mumias Sugar factory effluent disposal point and drains all the above stations and in addition, sugarcane cultures of Nzoia and Mumias Sugar factories, and Eldoret Municipality. Station 7 is on a tributary (approx. 3 m wide and 1.5 m deep) within the nucleus farm (3,400 ha.) of the Mumias Sugar factory. Herbicides are used almost exclusively to control weed in the nucleus farm. Station 8, 118,490 ha. watershed, drains all the above station in addition to the Kakamega municipality and other small-scale farms, which do not use herbicides. This accounts for about 90% of the total Nzoia watershed. Station 9, 129,690 ha. watershed, is the river delta where the river joins the lake through a swamp.

**Sampling**

Water (2.5 litres) and sediment (500 grams) samples were obtained from stations 1 - 9 between March 1998 and August 1998. Each site was sampled on at least 3 different days. The herbicides are applied pre-emergent in the maize and wheat fields before the annual pulse of the long rains occurring between April to June and in the sugarcane before the long or short rains (occurring in ca. October). All sampling stations (except stations 5 and 7) drain the preceding sampling stations on the river. Water was collected in precleaned amber bottles with Teflon-lined caps and the sediment was carried in black polyethylene bags. At all sites, samples were collected from approximately 15 cm depth of the river. Sediment was obtained by scraping the upper ca. 10 cm of the sediment at the bottom of the stream using a shovel. The samples were transported to the laboratory.
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within 24 h in cool-boxes. Sediment and acidified (HNO₃) water samples were stored in a freezer before later use.

Chemical analysis

Alachlor (molecular weight: 269.77, purity: 99%), metolachlor (molecular weight: 283.80, purity: 99%), 2,6-diethylaniline (molecular weight: 149.24, purity: >98%) and 2-ethyl-6-methylaniline (molecular weight: 135.21, purity: >97%) were obtained from Reidel de Häen Fluka (Seelze, Germany). Analytical grade n-hexane, dichloromethane and acetone were obtained from Sigma chemicals (Kenya). Anhydrous sodium sulphate was purified by soxhlet extraction with dichloromethane.

The extraction and cleanup procedures for water and soil samples were done according to Sánchez-Brunete et al. (1994) with some modification. In brief, excess water was discarded, leaving 2000 ml in the amber sampling bottles, to be subsequently mixed with saturated sodium sulphate (50 ml). Thereafter, the mixture was shaken with a dichloromethane:n-hexane (320 ml; 50/50 [v/v]) mixture for 5 min to extract the pesticides. After settling down, the supernatant was decanted into a 1-litre separating funnel and allowed to settle. The bottom layer comprising water was decanted off and the pesticide containing supernatant was subsequently cleaned and concentrated.

The sediment samples were dried at room temperature for 4 days and sieved (mesh size 0.5 mm) to obtain small-sized particles. Pesticides were extracted from the sediment (40 grams) by adding a mixture of acetone and n-hexane (40 ml, 50/50 [v/v]) (Funari et al. 1998). The extract was centrifuged (1000 rpm) for 20 min. The procedure was repeated and the clear supernatants were pooled in 200 ml flasks for a subsequent clean up and concentration procedures. Moisture contents of the remaining sediment samples were determined by deducing the differences between the oven dry (at 105°C for 2 h) and the room dry weights of the samples. The recoveries of the compounds under study were reported to be approximately 90% (Funari et al. 1998) and were not verified here. Thus, concentrations reported in this study were not corrected for recovery.

The extracts were cleaned up by passing them through a column (10 mm internal diameter) packed with preheated (210°C, 3 h) and cooled florisil (5 cm), and anhydrous granular Na₂SO₄ (5 cm), respectively. The
Column was eluted with diethyl ether (20 ml) and the extracts plus the eluate were concentrated by drying under a vacuum in a round-bottomed flask at 45°C. The products were reconstituted with n-hexane (5 ml) and stored in amber vials in a deep freezer. Analysis of the pesticides was performed using a Varian® Gas Chromatograph, model 3400CX equipped with an electron capture detector and a 30 m by 0.2 mm column fused with 0.2 μm silica film. An isothermal temperature program of 200°C, 250°C and 250°C for the column, injector and detector, respectively, was applied. Flow rate of the carrier gas (N₂) was maintained at 5 ml/min. Identification of alachlor, metolachlor, 2,6-diethylaniline, and 2-ethyl-6-methylaniline was based solely on their retention times, and the quantities of the compounds were deduced from the peak areas. The respective minimum detection limits for alachlor, metolachlor, and degradation products in water were 0.016, 0.004, and 0.008 μg/L and in sediment 0.198, 0.047, and 0.097 μg/Kg.

Concentrations of the pesticides and their degradation products

Fig 2 (A - D) shows chromatograms with peaks of alachlor (RT = 2.16 ± 0.002), metolachlor (RT = 2.63 ± 0.003), 2,6-diethylaniline (RT = 3.30 ± 0.003), and 2-ethyl-6-methylaniline (RT = 3.30 ± 0.003), respectively, in standard preparations (Fig 2A) and field samples (Fig 2B - 2D). Both 2,6-diethylaniline and 2-ethyl-6-methylaniline eluted at the same time. Thus, the test method proved not selective for the two degradation products, in agreement with previous reports (Tadeo et al. 1996). Degradation products occurred in all water and sediment samples except the station 1 sample (Fig 2B). Alachlor, metolachlor, and a probable combination of 2,6-diethylaniline and 2-ethyl-6-methylaniline were detected in 12.5, 2.1, and 93.8%, respectively, of the water and in 13.9, 2.8, and 97.2% of the sediment samples, respectively. The pesticides and their degradation products occurred at higher concentrations in the sediment than in the water samples (1.4 - 10800 fold) indicating an accumulation of the compounds in the sediment, Table 1. The degradation products were present throughout the entire sampling period inferring a stability of the compounds in the sediment or a continuous input of the pesticides into the river.
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Fig 2. Chromatograms of pesticides and degradation products A: mixture of standard chemicals, alachlor, metolachlor, 2,6-diethylaniline (dea), 2-ethyl-6-methylaniline (ema), B: station 1 water sample, C: station 9 water sample, D: station 9 sediment sample.
Dissipation of the pesticides and their degradation products

The relatively low detection frequency (<14%) of the parent compounds in the examined samples and the high detection rates of the degradation products (>90%) suggest that the parent compounds are metabolised quickly after application (Table 1). In a study conducted in a temperate agricultural catchment, alachlor and metolachlor were recovered from a higher percentage (>25%) of the water samples (Clark and Goolsby 2000). The movement of the pesticides and their degradation products from the field of application by leaching or runoff depends on their water solubility and partition-driven adsorption to the soil (Aga and Thurman 2001). Torrential rains, a characteristic of the tropics, generally lead to soil erosion, which may produce major pulses of the pesticides and their degradation products into surface water as both the soil adsorbed and dissolved organic compounds are moved along in the flash floods. Volatilizations of the compounds, ambient atmospheric temperature, and wind will each contribute to movement of the pesticides from the point of application to remote locations (Goolsby et al. 1997; Clement et al. 2000).

The solubility of alachlor, metolachlor, 2,6-diethylaniline and 2-ethyl-6-methylaniline in water at 25°C is 242, 488, 670 and 538× mg/L, respectively (Lyman 1982; Tadeo et al. 1996; Laabs et al. 2000), while their \( K_{oc} \) (octanol-carbon partitioning coefficient) are 312, 244, 357 and 197, respectively (Fava et al. 2000). Therefore, the degradation products are generally more soluble than the parent compounds, even though they bind more to the soil reducing their leaching potential (Gustafson 1989; Fava et al. 2000). The soil organic matter content and the nature of the organic matter affect the adsorption of the organic compounds on the soil, aromaticity of the humic material being the key structural parameter that regulates the sorption of the nonionic pesticides (Ahmad et al. 2001). The \( K_{oc} \) (octanol-carbon partitioning coefficient) values are strongly dependent on aromaticity and negatively correlated with alkyl carbon components (Ahmad et al. 2001).

In presence of sunlight and nitrate or dissolved humic acid, water is photolysed to liberate hydroxyl radical (\(^{•}\)OH) which degrades organic pollutants including the chloroacetanilides (Marbury and Crosby 1994; Brezonik and Fulkerson-Brekken 1998) thus enhancing the quantities of the

\(^{a}\) estimated by Hyperchem

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degradation products. It can be deduced from the high concentration of degradation products in the sediment (Table 1) that River Nzoia is already contaminated with the pesticide degradation products and that potential risk of higher contamination is substantial, especially with increased intensification of agriculture in the region. It is concluded that in our study the environmentally stable aniline metabolites of the pesticides and not the parent compounds were detected in the river water and sediment in consistence with the expected environmental behavior of the compounds in the area studied, given the higher ambient temperatures, the water regime and the agricultural practice.

**Risks of the pesticides and their degradation products in the tropics**

The tropical environmental conditions may enhance the toxicity of the chloroacetanilides and the risk of exposure to toxicants may be greater with higher temperatures, humidity and UV radiations all characteristics of the tropics (Viswanathan and Krishna Murti 1989; Blaustein and Wake 1995; Bossan et al. 1995; Abdullah et al. 1997; Heugens et al. 2001; Wiegmann et al. 2001). UV radiation, for instance, increases rate of frog malformation (Blaustein and Wake 1995) and UV may also weaken bonds between adsorbed chemicals thereby enhancing their bioavailability (Bossan et al. 1995). The parent and degradation products of the chloroacetanilides were acutely toxic to *Vibrio fischeri*, genotoxic to *Vibrio fischeri* (Mutatox® test), teratogenic to *Xenopus laevis*, and promutagenic in the Ames test (Kimmel et al. 1986; Osano et al. 2002b; Osano et al. 2002a). Therefore, both the parent and the degradation products are toxicologically important, however, in our study the degradation products proved more important pollutants of the river than their parent compounds. The standards set for water quality analyses of pesticide contamination have hitherto underrated the importance of the chemicals' degradation products. Our study showed values for the degradation products that exceeded the European Economic Commission limit of 0.1 and 0.5 μg/L set for any individual compound and total pesticides for drinking water, respectively (EEC 1998), and a lower value for the parent compounds in most samples. This finding differs from those in the temperate regions, where both the degradation products and parent compounds are found as environmental contaminants (Galassi et al. 1996). In view of toxic effects of the degradation products and apparent
<table>
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*Values represent averages of measured water and sediment samples.*
propensity for their formation in the tropics, we propose that risk assessments and water quality analyses in the tropic should routinely include pesticide degradation products.

References


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