Plasma levels of DDE/DDT and liver function in malaria control personnel 6 months after indoor residual spraying with DDT in northern Uganda, 2008

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Twenty million Ugandans are debilitated each year by malaria. Based on available evidence, and in the absence of a better and affordable alternative, DDT will continue to be used for disease vector control, as recommended by the World Health Organization (WHO), while further research clarifies the health impact of its use by indoor residual spraying (IRS).

Government policy therefore requires that DDT use should include assessment of its accumulation in the environment and an evaluation of its deleterious effects on man and the food chain. Consequently, it is necessary to record the applicators’ general state of health and the amounts of insecticide accumulated during and after IRS, to provide occupational-hazard values for corrective mitigation and for referral in subsequent studies. These data enhance national active information collection and reporting on the use of DDT for disease vector control.

Although animal models report dichlorodiphenyl-dichloroethylene (DDE)/dichlorodiphenyltrichloroethane (DDT) as a possible carcinogen, no such effects have been reported in humans. However, controversy exists concerning the liver: whereas some studies have reported impaired liver function, others reported an increase in the activity of liver enzymes, which could indicate liver disease such as hepatoma, or the usual enzyme catabolic induction typical of aromatic compounds, with no liver disease.

Our objectives were to determine the concentration of DDE/DDT in plasma samples from the spray team 6 months after their last spraying activity in northern Uganda in 2008, and to analyse plasma levels of biochemical markers of disease in blood from the spray team after exposure to DDT, so as to document acute/mid-term toxicity to internal organs, and especially liver function.

Methods

Approval for the study was granted by the Makerere University Faculty of Medicine Ethical and Research Committee. The project areas included Apac and Oyam districts and Lango sub-region (referred to as northern Uganda). Six months after the one IRS round of DDT in northern Uganda, the Ministry of Health’s Malaria Control Programme clinician reviewed and physically examined the DDT spray team after obtaining informed and signed consent. Venous blood samples (of 5 ml) were collected into heparin
Vacutainer tubes and delivered in cool boxes to the Department of Pathology, College of Health Sciences, Makerere University, for analysis for DDT and biochemical disease markers.

Laboratory procedures

The DDT in plasma was extracted with methanol and analysed using enzyme-linked immunosorbent assay (ELISA) kits. The samples were processed according to the manufacturer’s standard operating procedures and quality assurance instructions; during the reactions, colour that was inversely proportional to the concentration of DDE (the principal derivative of DDT) in the sample, developed; its intensity was translated and converted into concentration by comparison with that produced by the standards and controls supplied with the DDT reagent kits. The concentrations were read at 450 and 630 nm and printed automatically by the ELISA plate reader, the Stat FaxReg303 Plus.

Automated routine clinical chemistry methods were used in assaying for biochemical disease markers. Heparinised plasma samples were analysed for the concentration values of the selected biochemical markers of disease in plasma samples from spray applicators 6 months after the one round of DDT spraying.

Results

No abnormalities were detected on clinico-physical examination. No plasma abnormalities such as haemolysis, turbidity, chylosis, xanthochromia or jaundice were seen on visual inspection. The quantitative analytical results are presented at two levels: the primary level displays the main toxicological measure of interest (the DDT/DDE concentration in plasma samples from spray applicators 6 months after the one IRS round of DDT), and the secondary level displays the concentration values of the selected biochemical markers of disease.

DDE/DDT concentration in post-spray plasma

A total of 96 post-spray plasma samples were analysed for DDE/DDT concentration 6 months after the one round of DDT spraying (distribution shown in Fig. 1).

The DDE/DDT concentration in the post-spray plasma ranged empirically from 24 to 128, with a mean (SD) of 77 (26) ppb; the spread was near normal but leptokurtic and positively skewed; as was expected, there was DDE/DDT in every post-spray plasma sample analysed. The geometric mean was 71.81 ppb.

Biochemical markers of diseases of the liver and internal organs

A total of 119 plasma samples were analysed for the enzymes alpha-amylose, AST, ALT and GGT 6 months after spraying to screen for acute/mid-term toxicity of DDE/DDT in the internal organs – the liver, pancreas, heart and biliary tract.

Alpha-amylose concentration in post-spray plasma

In 119 post-spray plasma samples analysed for alpha-amylose, the mean (SD) enzyme activity concentration was found to be 71.86 (34.07) with a range of 16.80 - 199.50 µg/l, as shown in Fig. 2.

The distribution was polymodal and within the reference range of up to 300 µg/l. There was no alert value for any internal organ disease, especially not for acute pancreatitis as detected by plasma a-amylose in the post-spray plasma.

AST concentration in post-spray plasma

For post-spray AST activity concentration, 119 plasma samples were analysed; the mean (SD) enzyme activity concentration was found to be 450 and 630 nm and printed automatically by the ELISA plate reader, the Stat FaxReg303 Plus. The concentrations were read at 450 and 630 nm and printed automatically by the ELISA plate reader, the Stat FaxReg303 Plus. The distribution was expectedly leptokurtic and positively skewed (Fig. 3).

The distribution was polymodal and within the reference range of up to 300 µg/l. There was no alert value for any internal organ disease, especially not for acute pancreatitis as detected by plasma a-amylose in the post-spray plasma.

Fig. 1. DDDT concentration in post-spray plasma.

Fig. 2. Alpha-amylose enzyme activity concentration in post-spray plasma.
5 times the upper reference limit; there was no alert value for any internal organ disease, and specifically no hepatocellular or muscle damage, detected by high AST levels in the post-spray samples.

**ALT enzyme activity concentration in post-spray plasma**

In screening for acute/mid-term DDT toxicity in internal organs such as the liver, 119 plasma samples were analysed for ALT, which resulted in a mean (SD) of 7.80 (5.01) and a range of 1.00 - 17.00 µg/l. As expected, the spread was leptokurtic and positively skewed (Fig. 4).

The distribution was entirely within the reference range of up to 40 µg/l, with no internal organ disease indicated or hepatocellular damage detected by high levels of ALT in the post-spray samples.

**GGT enzyme activity concentration in post-spray plasma**

For screening for internal organ disease, for liver disease in general and for biliary obstruction in particular, 119 plasma samples were analysed for GGT enzyme activity concentration. The mean (SD) values were 58.37 (62.68) and from the range 12.50 - 333.40 µg/l. The spread was expectedly leptokurtic and positively skewed (Fig. 5).

Of the results, 75% were within the reference range of <60 µg/l; 30 were within the drug-inducible range of below 5 times the upper limit of the reference range; and there was no liver disease or biliary tree abnormality overtly detected by high GGT levels in the post-spray plasma samples.

**Discussion**

Our study found that DDT successfully protected against mosquito bites, with no health complaints emerging from the clinical history evaluation. We documented a DDE/DDT plasma arithmetical mean of 77 ppb, with no biochemically alarming concomitant mean values (such as alpha amylase – 66.94, AST – 18.18, ALT – 9.33, and GGT – 31.05 µg/l), indicating no disease associated with absorbed pesticide subsequent to IRS operations. These results refute the association of DDT with acute or mid-term diseases of human internal organs, especially of the liver; they confirm the safety of DDT as used in IRS operations, and strongly commend the use of DDT for disease control according to WHO guidelines, especially where there are no better and readily available alternatives.

The ELISA technology used in this study was highly recommended for pesticide analyses, especially in developing countries, where it offers the advantages of cost-effectiveness, simplicity and speed over chromatographic methods.7

According to the Abraxis test kit manufacturers, the DDE/DDT assay kit used in this study in particular had cross-reactivity to organochlorine compounds at different percentages as follows: pp’DDE – 100, pp’DDD – 46, op’DDD – 16, pp’DDT – 10, and op’DDE – 3.2. Whereas the method largely measured DDE (a metabolite of DDT), it did not appreciably measure DDT, and blood levels of DDE were, as usual, largely a measure of biotransformed DDT dependent *inter alia* on liver function and population genetics. We report and discuss them as DDE/DDT complex.
In our study, with the empirical range of AST being 0.70 - 69.90 µg/l, 12% of the plasma samples had values above the upper reference limit of 40 µg/l. Similarly, with the empirical range of GGT being 12.50 - 333.40 µg/l, 25% of the samples had GGT above the population reference limit of 60 µg/l but within the aromatic drug/alcohol inducible range for the enzyme.

We caution that laboratory results should not be considered in isolation: clinical history and physical examination should ideally precede laboratory investigations. Our mild increases in enzyme activities may be explained as follows: besides enzyme induction caused by DDT as a xenobiotic, the AST mild increase was attributed to the muscular exercise involved in the spraying activity; that of amylase was the result of the starch-rich millet-cassava diet made more prevalent by spray wages, and the GGT was because of increased consumption of lira-lira, a potent gin which is very popular in Northern Uganda.

This study in Northern Uganda in 2008, 6 months after only one round of DDT spraying, should for the first time provide the WHO with the essential information previously deemed missing, to evaluate exposure/response relationships to produce credible risk assessments of DDT use in IRS malaria control programmes.

References


5. Guzman PS. Clinical evaluation of liver structure and function in humans exposed to halogenated hydrocarbons. Environ Health Perspect 1985; 60: 159-164.


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