DDE-Induced Apoptosis in Children Exposed to This DDT Metabolite.

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Introduction.

DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] was used worldwide in agriculture and for the control of malaria; therefore, it is not unusual that levels higher than normal of DDT and its metabolites have been found in the environment[1] and in human tissues[1,2,3,4]. Recently, the Stockholm Convention on Persistent Organic Pollutants which came into force on 17 May, 2004, outlawed the use of 12 industrial chemicals including DDT[5]. However, one exemption clause allows malaria-endemic nations to use DDT strictly for indoor residual wall spraying.

DDT-induced health effects are still debated, as human data of DDT toxicity is somewhat limited; furthermore, regarding children's health, the information is even more scarce. In children exposed to DDT two kind of effects have been reported: neurological[6] and immunodeficiency[7,8]. In order to increase the knowledge, our group have been studying the effects of DDT in humans, such as apoptosis[9] and DNA damage[10]. For example, we have demonstrated that o'p-DDT, p´p-DDT, p´p-DDE and p'p-DDD are able to induce apoptosis *in vitro* in Peripheral Blood Mononuclear Cells (PBMC)[9], through the production of reactive oxygen species[11]. In addition, a preliminary association between the percentage of apoptotic cells and the levels of DDT and its metabolites in blood of exposed children was reported in a pilot study[9]. Taking into account that in several countries children are still exposed to DDT and its metabolites, and considering that the apoptotic properties of this insecticide could be a health issue for the exposed population, we expanded our studies in exposed children.

Materials and Methods.

Population. In order to obtain differential magnitudes of DDT exposure, three communities with history of indoor DDT spraying were selected. During the year 2003 we studied a total of 61 healthy children (aged 6-12 years). Samples of peripheral blood were collected into heparinized bottles. An aliquote was used for the TUNEL assay for apoptosis detection and the rest of the sample was immediately frozen and kept at -30°C until analysis for DDT, DDD and DDE.

DDT analysis in blood. DDT and its metabolites were quantified in blood using gas chromatography coupled with an electron capture detector as previously described[1].

TUNEL assay. For the analysis of apoptosis, heparinized blood from the exposed children was used to isolate Peripheral Blood Mononuclear Cells by Ficoll-Hypaque density gradient centrifugation. DNA fragmentation was detected by TUNEL assay using the APO-DIRECT detection kit (Pharmingen, San Diego CA), according to the manufacturer's instructions[9].

Results and Discussion.

Table 1. shows blood levels of DDT and its metabolites in children living in three communities located in South Mexico. We detected DDE in all children studied, DDE is the metabolite with the highest persitence. Apoptosis frequencies in PBMC in these children ranged from 0.10% to 8.30% (Table 2). However, a significant correlation was found only between apoptosis and DDE blood levels (p= 0.010 and 0.040).

The most important result in this work is that apoptosis was associated to DDE exposure, and this was showed in children exposed to different concentrations of DDE. This finding could implicate a health risk considering the chronicity of the exposure to DDE and the potential effects of apoptosis in the immune system. It is important to remember that apoptosis plays a variety of important roles under normal physiological conditions, but when it is out of regulation, apoptosis can contribute to immunodeficiency[12,13]. It seems evident that the uncontrolled elimination of immune cells may account for immunosupression or immune dysregulation[13,14]. In this regard, it has been reported that DDT-treated rodents showed different manifestations of immunodeficiency, including defective humoral and cell mediated immune responses[15,16]. Furthermore, effects in the immune system have also been reported in humans exposed to DDT or to its metabolites[7,8]. These studies provide evidence that DDE is able to modulate the immune system in children. Thus, as in this work, we have detected DDE-induced apoptosis of PBMC in exposed children, the effect of this metabolite on the immune system as well as its consequences on the susceptibility to infectious diseases needs to be adequately evaluated in the highly exposed populations of Mexico.

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Table 1. Blood Concentration of DDT, DDE, DDD and Total DDT in Children Living in Malarious Communities of South Mexico.

Compound	Community	n	mean	S.E.	Ranges
p'p-DDT	Ramonal, Q.Roo	23	14.62	12.88	2.80-46.22
	Cigüeña, Chiapas	16	5.44*	6.49	1.82-26.98
	Ventanilla, Oaxaca	22	6.76*	6.51	0.82-22.78
p'p-DDE	Ramonal, Q.Roo	23	52.48	43.61	14.13-182.60
	Cigüeña, Chiapas	16	46.77	23.60	20.06-104.46
	Ventanilla, Oaxaca	22	36.31	34.61	9.14-143.36
p'p-DDD	Ramonal, Q.Roo	23	4.16	5.76	nd-22.92
	Cigüeña, Chiapas	16	3.00	4.24	nd-18.36

	Ventanilla, Oaxaca	22	1.99*	2.62	nd-6.30
Total-DDT	Ramonal, Q.Roo	23	71.26	55.60	16.99-241.66
	Cigüeña, Chiapas	16	55.21	28.16	9.96-140.86
	Ventanilla, Oaxaca	22	45.06*	42.24	27.98-172.32

Blood concentrations are shown in µg/L. Values are geometric means. (*) p< 0.05 when compared to El Ramonal, Q. Roo. (S.D.) standar deviaton. (nd) no detected. The limit of detection for p'p-DDD was 0.048 µg/ml.

Table 2. Percentage of Apoptotic Cells in Blood of Children Exponed to DDT.

Community	n	mean	S.E.	Ranges
Ramonal, Q.Roo	23	1.91	0.45	0.37-8.28
Cigüeña, Chiapas	16	1.38	0.31	0.13-5.84
Ventanilla, Oaxaca	22	1.18*	0.30	0.10-2.77

The percentaje of apoptosis in blood cells was evaluated using the TUNEL assay. (*) p< 0.05 when compared to El Ramonal, Q. Roo.