Limited effect of selected organic pollutants on cytokine production by peripheral blood leukocytes

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Accepted for publication March 6, 2004

ABSTRACT. To test the hypothesis that some persistent organic pollutants contribute to the increased prevalence of allergic disease, the effects of selected compounds on cytokine production by PBMC from control and allergic donors were evaluated. Cells were cultured for six days in the presence of a xenobiotic (PCB 153, hexachlorobenzene, pentachlorobenzene, pentachlorophenol, lindane, atrazine or DMSO vehicle) with phytohemagglutinin (PHA) or *Dermatophagoides pteronyssinus* extract, then for one day in the presence of PHA + phorbol 12-myristate 13-acetate. PCB 153 reduced the levels of IL-10, IFN-γ and TNF-α. Hexachlorobenzene reduced the levels of IL-5, IL-10 and IFN-γ. Pentachlorobenzene reduced IL-6 levels. Pentachlorophenol reduced IL-5 levels. Lindane and atrazine reduced both IL-5 and IFN-γ. In addition, lindane reduced TNF-α levels. As these compounds had similar effects on cells from allergic and non-allergic donors, and as these effects were, in all cases, very limited indeed, the potential deleterious impact of the xenobiotics tested on the allergic response seems unlikely.

Keywords: house dust mite, *in vitro*, lindane, pesticide, pollution, polychlorinated biphenyl, T-helper, xenobiotic

INTRODUCTION

Our research is focused on the possible contribution of organic pollutants to immune responses in general, and to allergic responses in particular. Allergy is clearly multifactorial, but epidemiological data indicate that pollution plays a role in the development and progression of allergic diseases [1, 2]. The issue however, is very complex, with some forms of exposure being linked to development and exacerbations of the disease and others rather correlating with protection [3, 4]. Urban pollution negatively influences asthma and other allergic illnesses. However, the incidence of atopic asthma is lower in some heavily polluted areas [2, 5]. Recently, we have found that exposure to higher levels of dioxin-like compounds in adolescents was associated with a lower prevalence of upper airway disease [6]. Also, in Dutch preschool children, a history of higher exposure to dioxins correlated with lower prevalence of allergic diseases [7]. Experimental studies have contributed limited information only and have dealt mainly with heavy metals and with air pollution, in particular ozone, NO_x, SO_x and diesel exhaust particles [8, 9]. We have further tested the hypothesis that some common, persistent, organic pollutants modify the immune response. We had previously shown that the triazine herbicides atrazine and simazine, at micromolar concentrations, are responsible for a dramatic reduction in cytokine production by PBMC from healthy donors *in vitro* [10, 11]. The use of atrazine has been related to allergic symptoms in farmers, but the role of confounding variables could not be excluded [12, 13]. We have extended our studies to include several other persistent organic pollutants and adapted the culture protocol to favor expansion of allergen-specific cells. Therefore, we have examined cytokine production (Th1, Th2 and inflammatory cytokines) in leukocyte cultures obtained from allergic and non-allergic donors exposed *in vitro* to different test compounds including pesticides (atrazine and lindane); a non-dioxin-like polychlorinated biphenyl: PCB 153 and three other organochlorines: pentachlorophenol (PCP); hexachlorobenzene (HCB) and pentachlorobenzene (pentaCB). With the exception of pentaCB, all these compounds have an established or suspected record of hemato-immunotoxicity. Data available so far were mainly obtained from subjects exposed *in vivo* (e.g. professionally), usually to mixtures of pollutants or from animal studies (references for atrazine [10-12]; for lindane [13-15]; for PCBs [16-19]; for HCB [19-24] and for PCP [25-28]). The compounds, belonging to different classes of persistent pollutants, were selected for study in view of their relevance for industrialized as well as developing countries. Even if some of them have been banned, or if their use is being strongly discouraged, they are still present in the environment: atrazine and lindane are still present at detectable levels in drinking water from many countries [29,30]; lipid-soluble PCBs and HCB contaminate the food chain [31]. Significant exposure to PCP occurs in the general population through leather, textiles and, most of all, wood [32].

DONORS AND METHODS

Study subjects

Control donors had a negative history and no positive RAST test. The study was approved by the Ethical Committee of the Academic Hospital. We obtained blood from 44 adult volunteers, personnel or students from the Medical School and the Academic Hospital of the V.U.B. (see *Table 1* for characteristics of the donors). All donors live in suburban Brussels and are not exposed professionally to the compounds tested. Twenty four of them had an established allergic disease (atopic asthma, rhinitis, conjunctivitis or dermatitis), and at least one positive RAST test. Of these 24 allergic subjects, 16 had a positive RAST for Dpt and 14 of these were also allergic to at least one mixture of allergens. The remaining eight allergic subjects reacted to one mixture (three donors) or to several mixtures of allergens (five donors). Twenty donors were non-allergic (negative anamnesis and all six RAST tests negative).

Reagents

Phytohemagglutinin (PHA, Reagent grade HA 15) was obtained from Murex (Dartford, Kent, UK), *Dermatophagoides pteronyssinus* extract (Dpt, Lyophilized allergen ZN) from HAL Allergenen Lab (Haarlem, The Netherlands). 2,2', 4,4', 5,5'- Hexachlorobiphenyl (PCB 153), and atrazine were from Riedel-de Haën (Seelze, Germany). γ-Hexachlorocyclohexane (lindane), pentachlorobenzene (pentaCB), hexachlorobenzene (HCB), pentachlorophenol (PCP) and phorbol 12-myristate 13-acetate (PMA) were from Sigma-Aldrich (Bornem, Belgium).

PBMC cultures

The technique has been adapted from Moverare *et al.* [33]. Venous blood (40 mL) was taken over heparin in Vacutainer tubes (Becton Dickinson, Erembodegem, Belgium). After a plasma sample was removed for RAST tests and total IgE determination, PBMC were separated from heparinized blood by centrifugation through Ficoll-Paque (Lucron Bioproducts, De Pinte, Belgium). After two washes in Hanks' buffered saline solution without Ca^{++} and Mg^{++} (Invitrogen, Paisley, UK), and one wash in RPMI 1640 supplemented with antibiotics (100 units/mL Penicillin G sodium, 100 µg/mL streptomycin sulphate and 250 ng/mL amphotericin B), the cells were resuspended in RPMI supplemented with 10% heat-inactivated human AB serum "off the clot" (PAA, Cölbe, Germany), 50 µM β-mercaptoethanol and antibiotics. PBMC were cultured at 37 °C in a humidified, 5% CO_2 atmosphere in 24-well, flat-bottomed cell culture plates (Nunc, Denmark) at 2×10^6 living cells/mL. Medium was either plain or supplemented with 2 µg/mL PHA or with 10 µg/mL Dpt extract. Cells from all donors were stimulated with Dpt extract as the allergic status towards Dpt was not firmly established at the time of sampling and start of culture. In addition, pollutant (pentaCB, HCB, lindane, PCB 153 or PCP, all at 100 nM or atrazine at 3 µM) in DMSO or DMSO vehicle only, was added at the start of the culture. In all cases, the final concentration of DMSO was 0.01%. After six days, the plates were centrifuged, medium was removed and stored at – 20 °C. Cells were washed, fresh medium was added and the cells were re-stimulated with 10 µg/mL PHA and 10 ng/mL PMA. After 24 h, the supernatants were removed and frozen (– 20 °C) until the cytokine analysis was performed. As a consequence of variable cell yields, not all experimental conditions could be tested on all donors. Results are presented only when at least 10 samples (five from allergic and five from non-allergic donors) were available for testing.

Cytokine assays

Concentrations of cytokines in cell culture supernatants were measured by commercial ELISAs for IFN-γ, TNF-α, IL-4, IL-6, IL-10 (BioSource Europe, Nivelles, Belgium), IL-5 (Pharmingen, Erembodegem, Belgium) and IL-13 (Pelipair, Central Laboratory of the Netherlands Red Cross, Amsterdam, The Netherlands). The detection limit was 4 pg/mL for all cytokines except for IL-13 (1 pg/mL) and for IFN-γ (10 pg/mL). Through lack of material, not all cytokines could be tested in all samples.

Statistics

Statistical analysis was performed with GraphPad Instat version 3.00 for Windows 2000, GraphPad Software, San Diego California USA, www.graphpad.com. Cytokine levels in cultures grown in unsupplemented medium or in medium supplemented with PHA or with Dpt extract were compared using the Tukey-Kramer multiple comparison test. For each donor, the cytokine concentration measured in the presence of a given pollutant was calculated as the%

Table 1

Study population	*N*	Age	M/F	IgE ng/mL	Dpt. kU_A/L
All donors	44	35	13/31	207 ± 479	
Allergic donors	24	34	8/16	304 ± 629*	
Allergic to Dpt	16	33	5/11	414 ± 752**	26 ± 25
Mono-allergic[1]	2	26	1/1	124	25
Poly-allergic	14	34	4/10	455 ± 799	28 ± 28
Non-allergic to Dpt	8	35	3/5	84 ± 91	
Mono-allergic	3	43	1/2	52 ± 48	
Poly-allergic	5	30	2/3	103 ± 110	
Non-allergic donors	20	36	5/15	92 ± 118	

* IgE levels in allergic donors significantly higher ($P = 0.01$, 2-tailed Mann-Whitney test) than in non-allergic donors.
** IgE levels in donors allergic to Dpt significantly higher ($P = 0.001$, 2-tailed Mann-Whitney test) than in non-allergic donors.
[1] Mono-allergic refers to symptomatic donors with only one positive RAST test (out of at least six performed). Poly-allergic means symptoms + at least two positive RAST tests.

of the control cultures without pollutant. In the case of IL-4 and IL-10, values were often below the detection limit, but there was no case were values became detectable or undetectable as a result of exposure to pollutant. Whenever values were below the detection limit for a given cytokine, the effect of a pollutant on its production could not be analysed. The paired t test was performed and the 2-tailed *P* values were calculated to assess the effects of pollutants using GraphPad InStat.

IgE plasma levels

Total IgE levels were determined in plasma using the Elecsys system (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's guidelines. As expected, the total plasma IgE levels were higher in allergic subjects than in non-allergic subjects (304 ± 629 µg/L *versus* 92 ± 118 µg/L; $P = 0.01$, Mann-Whitney 2-tailed test, see *Table 1*).

Rast

RAST tests were performed for Dpt and for mixtures of allergens from food (fx5), grasses (gx3), trees (tx5 and tx6) and epithelia (ex1) using UniCAP specific IgE fluoroenzyme immunoassays (Pharmacia Diagnostics, Uppsala, Sweden), according to manufacturer's guidelines. In addition, if allergy to another allergen was reported, RAST for this particular allergen was performed as well. Values above 0.70 kU_A/L (for Dpt) or above class 1 (for mixtures) were considered positive.

RESULTS

In vitro studies with cells from allergic subjects commonly involve a six- or seven-day culture to expand allergen-specific cell populations, poorly represented in the starting sample [29]. We have thus cultivated cells (in medium either unsupplemented or supplemented with PHA or with Dpt extract) and collected supernatants after six days. Fresh medium, containing PHA and PMA was then added for 24h. Very low, mostly undetectable levels of IFN-γ, IL-4 and IL-10 were found after six days, whereas TNF-α, IL-5, IL-6 and IL-13 could be detected. At day seven, IL-4 and IL-10 were still undetectable in a certain proportion of samples. Cytokine levels are presented in *Table 2* (cultures without pollutants), and *Table 3* (effect of pollutants, mean of n individual values each expressed as a percentage of culture without pollutant). Only the mean IL-5, IL-13 and TNF-α levels were significantly increased in PHA-treated cultures (compared to unsupplemented cultures) without xenobiotics. For the different cytokines, there were no significant differences between allergic and non-allergic donors. At day six, only two donors allergic to Dpt (out of 13 tested) reacted to the allergen with high IL-5 production (above 100 pg/mL). For other donors allergic to Dpt as for all other donors (allergic to other allergens or not allergic at all), the response to PHA was higher than the response to Dpt extract.

Hexachlorobenzene and Pentachlorobenzene

Cells grown in the presence of HCB produced, after re-stimulation with PHA + PMA, less IFN-γ and IL-10 and, only when stimulated with PHA, less IL-5. The reduction of IL-5 levels was greater in samples from allergic donors (see *Figure 1* and footnote to *Table 3a*). The related substance pentaCB had no significant effect on cytokine production, with the exception of a lower IL-6 production in cells stimulated with PHA.

PCB 153

PCB 153 lead to decreased levels of IL-5 (at day six) and of IFN-γ, TNF-α and IL-10 (at day seven). All these effects were seen in Dpt- (but not in PHA-) stimulated cultures.

PCP, lindane and atrazine

Chlorinated pesticides (PCP, lindane and atrazine) were responsible for decreases in IL-5. In addition, IFN-α was reduced with lindane and atrazine, and TNF-γ production with lindane. The effects of lindane were seen only after stimulation with Dpt extract, whereas those of atrazine were apparent only in PHA-stimulated cultures.

DISCUSSION

Studies on cytokine production yield important information on mechanisms of normal and pathological immune responses. *In vitro* cytokine production is also a useful indicator during toxicological evaluation [18, 19, 24-26].

After a six-day culture, PHA and Dpt extract stimulated cytokine production to various extents. Further stimulation with PHA and PMA resulted in the release of measurable amounts of IFN-γ, TNF-α, IL-5, IL-6, IL-13 and, in most cases, IL-4 and IL-10 too. Culture conditions during the first six-day period had little impact on the cytokine release during the seventh day, except for IL-5 and IL-13: the production of these cytokines was clearly stimulated by a prior exposure to PHA (*Table 2, d7*, unsupplemented *versus* PHA *versus* Dpt extract).

The present work extends previous studies with atrazine. When PBMC from normal donors were grown for three days in the presence of atrazine (3 µM), the production of TNF-α, IFN-γ and IL-5 (but not IL-8) was greatly reduced. Similar data were obtained with another triazine herbicide, simazine [10, 11]. Also in the present study, atrazine was responsible for a decrease in cytokine production, but effects were less pronounced after seven than after three days. Although the experimental procedure followed in the present paper has been of great value in the study of the allergic responses, it may lack sensitivity for the detection of immunotoxic effects of a number of compounds. Atrazine is still the most commonly used herbicide in the U.S. and possibly in the world. In Hungary, for instance, the maximum detected residues of atrazine in stream water were one order of magnitude higher than the maximum residue limit specified by the European Union for environmental and drinking water (0.1 µg/L, i.e. about 5 nM) [29,30]. Although the EPA's current drinking water standard for atrazine is 3 µg/L (about 14 nM), atrazine at 0.1 µg/L (about 0.5 nM) induces hermaphroditism in male frogs [34].

The other pollutants were tested at 100 nM, a relatively high concentration for most compounds, except in the case of PCP where higher serum concentrations have been reported even in recent studies involving human subjects [35, 36]. This concentration was selected on the basis of literature data and preliminary experiments (not shown).

Table 2
Effect of PHA and Dpt extract on cytokine production by PBMC in culture (pg/mL) (allergic + non-allergic donors)

First stimulus[1]	0	PHA	Dpt
Cytokine			
IFN-γ (*n* = 35) d7	3 622 ± 5 187	4 422 ± 6 286	2 460 ± 2 373
TNF-α (*n* = 19) d6	238 ± 196	3 262 ± 4595*	3 303 ± 3 792*
TNF-α (*n* = 35) d7	8 127 ± 6 491	9 353 ± 8 647****	6 459 ± 8 271
IL-4 (*n* = 25) d7	13.2 ± 11.6 (*n* = 21)[2]	22.4 ± 23.0 (*n* = 22)	16.9 ± 13.4 (*n* = 21)
IL-5 (*n* = 28) d6	12.8 ± 2.6	66.8 ± 105.2*	47.7 ± 80.1
IL-5 (*n* = 43) d7	32.1 ± 34.7	182.7 ± 183.4***	47.1 ± 85.0
IL-6 (*n* = 10) d6	440 ± 416	3 912 ± 4 000*	40 819 ± 24 222**
IL-6 (*n* = 41) d7	900 ± 3473	795 ± 2 528	1 154 ± 3 410
IL-10 (*n* = 23) d7	16.8 ± 29.2 (*n* = 17)[2]	18.3 ± 26.9 (*n* = 17)	10.4 ± 17.9 (*n* = 17)
IL-13 (*n* = 13) d6	1.8 ± 0.3	49.9 ± 31.1***	17.7 ± 21.7
IL-13 (*n* = 42) d7	393.4 ± 304.4	1 531.2 ± 1 320.6***	471.3 ± 372.6

Supernatants were taken after a 6-day culture (d6) or a 6-day culture followed by 1-day restimulation with PHA + PMA (d7, see methods).
* $P < 0.01$ when compared to unstimulated cultures.
** $P < 0.0005$ when compared to either unstimulated or to PHA-stimulated cultures.
*** $P < 0.01$ when compared to either unstimulated or to Dpt-stimulated cultures.
**** $P < 0.01$ when compared to Dpt-stimulated cultures.
[1] First stimulus refers to the stimulus present during the first six days of culture. The second stimulus is PHA + PMA.
[2] For IL-4 and IL-10, the n value in the first column gives the number of samples tested. The n values given in the other columns represent the mean ± SD of samples above the detection limit. For other cytokines, all values were above the detection limit.

Lindane, another pesticide that was commonly used, induced, in cells stimulated with Dpt extract, a decrease in IFN-γ and TNF-α levels. Lindane was tested in view of its persistence in the environment including drinking water [30]. There is still controversy surrounding the immunotoxicity of lindane, because the material used in studies showing a very high immunotoxicity (which would be the most sensitive, non-carcinogenic end-point for this compound), did not meet the present criteria for purity, and the toxicity could possibly be due to the contaminants.

Hexachlorobenzene also reduced IFN-γ, IL-10 and IL-5 levels. The latter was the only observation that could be specifically related to the allergic status, as it was not seen in non-allergic donors. A reduced production of IFN-γ by PBMC from workers professionally exposed to HCB (and having plasma levels above 1.1 μg/L HCB) had been reported previously. No reduction in Th2 cytokines was found and a specific impairment of the Th1 arm was thus proposed [37]. A decrease in IL-10 production has already been reported in Lewis rats exposed to HCB [24]. Since a decrease in IL-10 is the strongest effect that others and we observed, we suggest that this contributes to the increased inflammatory responses seen with HCB in several experimental systems [21-24, 38]. In Inuit infants exposed to a mixture of organochlorine pollutants, an increased incidence of middle ear infection was correlated with exposure to organic pollutants, in particular to HCB [39]. The parent substance pentaCB, which has a much lower toxicity record, was tested next to HCB and was indeed found to have even fewer effects on cytokine production, as only IL-6 production was found to be reduced.

After treatment with PCB 153, there were reductions in IFN-γ, IL-5, IL-10 and TNF-α. Reduction in TNF-α production had been previously reported after *in vitro* exposure to other PCBs (about 10^{-7} M) [18]. In a group of Flemish adolescents, a higher exposure to dioxins was correlated with lower IgE levels [6]. This is in line with the reduction in IL-5 that we observed with PCB 153. This compound is one of the most commonly detected PCB congeners in breast milk [17, 40]. Only a few immune parameters (IgG and IgM production) were altered in monkeys fed a mixture of PCB congeners (analogous to those found in breast milk) [41].

Of all the compounds tested, PCP has been most frequently linked to immunotoxicity. We only observed a reduced IL-5 production during the first six days in culture. In subjects exposed to PCP and with elevated blood levels of PCP, cellular and humoral deficiencies, as well as T-cell activation and autoimmunity have been reported [26, 28, 36]. Th1 and inflammatory, but not Th2 cytokine levels were reduced in serum, but cytokine production in culture was not examined [27]. Until recently, in adults, serum PCP concentrations of 100 nM and higher were not exceptional [27, 37].

Taken together, the present study confirms that some persistent pollutants can affect cytokine production *in vitro*. After six or seven days, effects of pollutants were very limited indeed and in most cases were seen in samples from allergic as well as from non-allergic donors. There were few differences between allergic and non-allergic donors, or among allergic donors themselves, or between subjects allergic or not to Dpt, even though such differences were carefully looked for. The allergen used in the present study induced very robust responses in a few donors, but failed to do so in several donors with a positive RAST for Dpt. Discrete effects were seen at concentrations that are similar or slightly above the concentrations found in human plasma in specific groups, though in non-exceptional conditions: professionally exposed workers, Inuit children, or even a considerable fraction of the general population. For several compounds, our data are in close agreement with observations made in a different setting, namely with cells obtained from exposed subjects and then cultivated in the absence of pollutants. Our data, however, provide very little evidence for a deleterious effect of any of the tested pollutants on the effector phase

Table 3
Cytokine production in cultures of PBMC from allergic + non-allergic donors treated with pollutants (percentage of production in untreated cultures) after 6- (IL-5) or 7-day cultures (all cytokines)

a. Hexachlorobenzene

First stimulus	PHA	Dpt
Cytokine		
IFN-γ (*n* = 26)	93.9 ± 12.9*	87.4 ± 21.2 *
TNF-α (*n* = 21)	99.4 ± 15.0	94.8 ± 14.0
IL-5 (*n* = 12) d6	94.3 ± 12.7	89.4 ± 18.1
IL-5 (*n* = 32) d7	93.8 ± 10.2*[1]	95.8 ± 16.9
IL-6 (*n* = 30)	93.2 ± 36.2	104.9 ± 44.8
IL-10 (*n* = 10)	81.5 ± 19.5*	90.8 ± 8.0*
IL-13 (*n* = 31)	95.4 ± 11.9	99.2 ± 17.7

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$
[1] effect significant in the pooled donor group (all donors, *n* = 32) and even more so in the allergic donors (92.1 ± 11.3**, *n* = 17) but not in the non-allergic group (95.7 ± 8.7, *n* = 15).

b. Pentachlorobenzene

First stimulus	PHA	Dpt
Cytokine		
IFN-γ (*n* = 10)	97.9 ± 17.7	103.0 ± 36.5
TNF-α (*n* = 10)	98.9 ± 15.3	100.6 ± 14.8
IL-5 (*n* = 10) d6	86.7 ± 38.3	110.7 ± 17.6
IL-5 (*n* = 10) d7	103.2 ± 17.3	101.9 ± 14.9
IL-6 (*n* = 10)	83.3 ± 20.5*	90.5 ± 41.3
IL-13 (*n* = 10)	100.6 ± 15.1	112.6 ± 50.2

* $P < 0.05$

c. PCB153

First stimulus	PHA	Dpt
Cytokine		
IFN-γ (*n* = 16)	99.8 ± 19.2	85.8 ± 20.3 *
TNF-α (*n* = 16)	104.4 ± 17.6	91.9 ± 10.4 ***
IL-5 (*n* = 10) d6	91.9 ± 20.5	86.1 ± 16.2*
IL-5 (*n* = 14) d7	105.3 ± 17.4	90.8 ± 20.4
IL-6 (*n* = 14)	86.1 ± 54.1	97.1 ± 36.9
IL-10 (*n* = 10)	90.1 ± 22.0	88.4 ± 4.7***
IL-13 (*n* = 15)	123.5 ± 51.4	94.8 ± 19.1

* $P < 0.05$; *** $P < 0.005$

d. Pentachlorophenol

First stimulus	PHA	Dpt
Cytokine		
IFN-γ (*n* = 12)	97.2 ± 12.6	98.8 ± 16.4
TNF-α (*n* = 10)	106.4 ± 17.6	98.4 ± 8.3
IL-5 (*n* = 10) d6	80.2 ± 22.3*	90.8 ± 21.7*
IL-5 (*n* = 11) d7	96.6 ± 8.8	106.6 ± 16.8
IL-6 (*n* = 11)	99.1 ± 53.6	105.4 ± 34.4
IL-13 (*n* = 11)	112.8 ± 30.9	98.1 ± 17.0

* $P < 0.05$

e. Lindane

First stimulus	PHA	Dpt
Cytokine		
IFN-γ (*n* = 18)	92.3 ± 16.3	90.7 ± 20.5*
TNF-α (*n* = 25)	95.6 ± 17.0	88.4 ± 18.8*
IL-5 (*n* = 10) d6	96.0 ± 13.2	80.9 ± 14.1**
IL-5 (*n* = 22) d7	95.5 ± 14.8	88.6 ± 24.6
IL-6 (*n* = 22)	84.8 ± 47.0	98.1 ± 47.4
IL-13 (*n* = 22)	89.8 ± 14.7	94.0 ± 19.6

* $P < 0.05$;** $P < 0.01$

f. Atrazine

First stimulus	PHA
Cytokine	
IFN-γ (*n* = 13)	87.7 ± 20.4*
TNF-α (*n* = 13)	90.6 ± 30.7
IL-5 (*n* = 10) d7	85.2 ± 10.7*
IL-6 (*n* = 11)	119.7 ± 43.1
IL-13 (*n* = 10)	93.6 ± 24.5

* $P < 0.05$

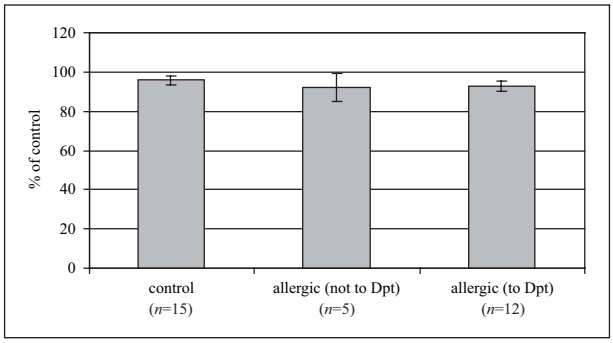

Figure 1
Effect of HCB on IL-5 secretion in control donors (*n* = 15), in allergic donors (not allergic to Dpt) (*n* = 5), and in allergic donors (allergic at least to Dpt) (*n* = 12).
Cells were exposed for six days to PHA, then one day to PHA + PMA (see Materials and methods). The histograms represent, for each group, the ratio (± S.E.) of IL-5 concentration in HCB-treated cultures over control (DMSO vehicle-treated) cultures. IL-5 concentrations in control cultures for the three groups were respectively: 195.7 ± 234.3, 110.4 ± 100.5 and 235.6 ± 260.8 pg/mL (mean ± S.D.).

of an allergic response. Mainly inhibitory effects on cytokine production were seen. However, reduced IL-10 levels (as observed with HCB and PCB 153) could favor an enhanced pro-inflammatory state, at least in the case of HCB where there was no concomitant reduction in TNF-α. There was no selective modulation of the Th1 or the Th2 response. This does not exclude the possibility of clinically relevant effects in other settings, for instance after exposure to mixtures of xenobiotics, or during the maturation of the immune system or in immunocompromised patients or during the sensitization phase [16, 33]. Indeed, two of our observations may point to a specific effect of pollutants on allergic responses: the effect of HCB on IL-5 was more pronounced in allergic donors (see *Figure 1* and footnote to *Table 3*) and effects of lindane and PCB 153 were seen in cells stimulated with the allergen but not in cells stimu-

lated with PHA. Both facts may point to a stronger effect of these compounds on certain cell populations, more specifically related to allergic responses.

ACKNOWLEDGEMENTS. This work was supported by a Prospective Research for Brussels grant, a grant from the Flemish Government (GOA 97-02-4) and by the V.U.B. Thanks to Daniella Ooms for excellent help with the ELISAs, to Johan Schietecatte for IgE determinations and RASTs, to Nick Allaerts for blood collection, to physicians for sharing information, to volunteers for giving blood and to Sarah Gerlo, Ron Kooijman and Jacques Van Snick for discussion.

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