



The relationship between persistent organic pollutants in maternal and neonatal tissues and immune responses to allergens: A novel exploratory study

Paul S. Noakes^a, Peter Taylor^b, Steve Wilkinson^b, Susan L. Prescott^{a,*}

^a School of Paediatrics and Child Health, University of Western Australia, Perth, Western Australia 6001, Australia

^b Chemistry Centre of Western Australia, Perth, Western Australia 6001, Australia

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Abstract

Background: Modern persistent organic pollutants (POPs) contamination are logical candidates in the investigation of the, as yet, unexplained association between allergic disease and progressive industrialisation. POPs have been detected in human cord blood, placental tissues and breast milk, and the reported association between cord blood IgE levels and cord/placental POP levels has raised concerns about potential immunological effects in early life.

Methods: The initial aim of this study was to determine if POPs were detectable in maternal blood, cord blood, placental tissues, adipose tissue and breast milk samples from randomly selected Western Australian women ($n = 31$), where allergic disease is epidemic. Gas chromatography was used to detect polychlorinated biphenyl compounds [PCBs] (as Aroclor 1232, 1254, 1260) and organochlorine (OC) pesticides, including *p,p'*-DDT, *p,p'*-DDE, hexachlorobenzene (HCB), lindane, heptachlor epoxide, dieldrin and chlordane. Secondly, we assessed the relationship between POP levels detected in vivo and maternal and neonatal responses (cytokine and lymphoproliferation) to allergens and mitogens.

Results: Low level POP contamination was detected in adipose tissue and breast milk (but not in cord blood, maternal blood or placental tissues). The most ubiquitous compound found in over 90% of adipose tissues samples was a OC metabolite of DDT, *p,p'*-DDE (median 0.07 mg/kg; interquartile range [IQR] 0.05–0.12). However, the majority of other OC compounds were not detectable and PCB were not detectable in any samples. The three main residues detected in breast milk were *p,p'*-DDE (0.003 mg/l; 0.001–0.009), dieldrin (0.001 mg/l; 0.001–0.046) and HCB (0.001 mg/l; 0.001–0.001). These levels are significantly lower than reported over 20 years ago. There were no consistent relationships between POP levels in vivo and maternal or infant responses, with the exception of a significant inverse association (Spearman rank correlation: $r = -0.406$, $p = 0.049$) between maternal adipose tissue levels of OC *p,p'*-DDE and maternal T helper cell Type 1 interferon [IFN] γ to mitogens.

Conclusion: This study provides the first evidence (in Australia) since the early 1990's that adipose OC levels have continued to fall. The negligible levels in this randomly selected group are significantly lower than those previously recorded, suggesting that POP contamination (at biologically relevant levels) is not likely to be a major contributing factor in the increasing rates of allergy in Western Australia. However, the relationship between Th1 immune function and OC contamination is consistent with other reports and is worth investigating as a relevant factor in populations where OC contamination is greater.

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* Corresponding author. Tel.: +618 9340 8171; fax: +618 9388 2097.

E-mail address: susanp@icmr.uwa.edu.au (S.L. Prescott).

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1. Introduction

The dramatic and costly increase in allergic diseases (such as asthma, allergic rhinitis, dermatitis and food allergy) over the last four decades has made it imperative to determine the contributing environmental changes. These are likely to be multifactorial, but while a number of candidate factors have been investigated (as yet inconclusively), there are virtually no studies to investigate the role of persistent organic pollutants (POPs) which have been new to our environment during the last century.

Increasing levels of previously nonexistent industrial and agricultural by-products have been among the most dramatic environmental changes with progressive industrialisation. Many classes of these persistent organic compounds (such as the polychlorinated biphenyl compounds [PCBs], dioxins, furans, and organochlorines [OCs]) contaminate modern homes, food, clothing and water sources, and have been shown to accumulate in human tissue with age (Nunes and Tajara, 1998; Ntow, 2001; Smeds and Saukko, 2001). POPs have documented immunological effects, and are immunosuppressive at high doses in humans (Daniel et al., 1995). Low levels of POP contamination have been shown to more selectively inhibit Type 1 immune responses (Daniel et al., 2001), leading to speculation that this could possibly favour allergic (Type 2) immune responses. Although no causal pathways have been identified, these more subtle pro-allergic effects could be mediated by the “oestrogenic” hormonal activity of many of these compounds. Effects are likely to be greatest on the developing immune system, and could potentially influence the pattern of subsequent responses. Of concern, various POPs have been measured in human cord blood, placenta and breast milk (Stacey et al., 1985; Stevens et al., 1993; Rhainds et al., 1999; Bjerregaard and Hansen, 2000; DeKoning and Karmaus, 2000; Ataniyazova et al., 2001). Moreover, one eastern European group noted (Reichrtova et al., 1999) that higher POP levels in cord blood (PCBs) were associated with higher levels of cord blood IgE antibodies. These observations highlight POPs as possible candidate factors in the “allergy epidemic” which has been associated with progressive industrialisation.

This novel exploratory study was designed to determine if POP levels are readily detectable in biological samples from randomly selected women living in Western Australia where allergic disease is epidemic, to determine if this is a likely or possible contributing factor that should be pursued in further studies. Our secondary aim was to examine the relationship between

levels of POP detected in vivo and maternal and neonatal immune responses.

2. Methods

2.1. Study subjects and samples collected

A cohort ($n = 31$) of randomly selected pregnant women booked for elective caesarean section at King Edward Memorial Hospital, Subiaco, Western Australia were recruited to evaluate POP levels in peripheral blood, cord blood, breast milk, placenta and abdominal fat. As the prevalence of allergy in the WA population is high (ISAAC, 1998), we did not select for maternal allergy in this birth cohort. Ethical approval for this study was granted by the Princess Margaret Hospital Research and Ethics Committee. All women signed a written consent form.

2.2. Sample analysis

All analyses were performed by an accredited laboratory [Chemistry Centre (WA)] using appropriate QA/QC methodology. All samples were subject to extraction and, where necessary, clean-up to remove interfering compounds and matrix before analysis by gas chromatography with electron capture.

2.2.1. Maternal and neonatal blood samples

All samples ($n = 29$ maternal and $n = 28$ neonatal blood) were analysed in June–July 2002 using the OCPCBBLOOD method (Pendleton et al., 2003). This method consisted of extraction of a blood sample using hexane and analysis of the extract using gas chromatography with dual column for confirmation and electron capture detection (Najam et al., 1999).

2.2.2. Human milk analysis

The milk samples ($n = 21$) were analysed in February 2003 using the ASEMILK method. The milk samples were adsorbed onto a non-reactive matrix and the relevant analytes were extracted using a modified accelerated solvent extraction (ASE) procedure. The extracts were cleaned up during the extraction procedure and analysed by gas chromatography with dual columns for confirmation and electron capture detection (Luke et al., 1985/86; Nakagawa et al., 1999; Yague et al., 2001).

2.2.3. Tissues samples

All adipose tissue and placental tissue ($n = 31$) samples were analysed between December 2002 and Febru-

ary 2003 using OCPCB in Biota method. This method consisted of the extraction of the homogenised tissue sample into hexane. The analytes were then separated from the protein and fat by gel permeation chromatography and the resulting solution analysed by gas chromatography with dual columns of the confirmation and electron capture detection (Armishaw and Millar, 1998; Weichbrodt et al., 2000).

These methods detected OCs at a sensitivity of 0.02 mg/kg for tissue samples; 0.001 mg/l and 0.002 mg/l for whole blood and breast milk samples, respectively. Two of the adipose tissue samples had higher quantitation limits due to insufficient amount of tissue and the MQLs for these samples was 0.03 mg/kg and 0.05 mg/kg. Detection limits for PCBs in tissue and blood was 0.3 mg/kg and 0.01 mg/l, respectively.

2.3. Gas chromatographic analysis

A gas chromatograph (Varian 3800) equipped with two ^{63}Ni electron capture detectors was used for the analysis. Dual capillary GC columns (J&W DB-608 30 m $\times$ 0.32 mm ID $\times$ 0.5 μm film thickness and Restek RTX-CLPesticides 30 m $\times$ 0.32 mm ID $\times$ 0.5 μm film thickness) were used for the OC pesticides. Working conditions were: injector 260 $^{\circ}\text{C}$ (2 μl injection), detector 340 $^{\circ}\text{C}$, and column 60 $^{\circ}\text{C}$ for 0.5 mins, 50 $^{\circ}\text{C}/\text{min}$ to 170 $^{\circ}\text{C}$, hold 0.5 min, 10 $^{\circ}\text{C}/\text{min}$ to 280 $^{\circ}\text{C}$.

2.4. Persistent organic pollutants

Residues of 20 OC compounds in maternal and neonatal tissue samples were analysed:

2.4.1. Organochlorine insecticides

Aldrin; chlordane (a- and b-); dicofol; dieldrin; 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*o,p'*- and *p,p'*-DDT); 1',1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE); 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDD); endrin; endosulfan (a- and b-); endosulfan sulfate; heptachlor; heptachlor epoxide; hexachlorobenzene (HCB); lindane; oxychlordane; tetradifon.

2.4.2. Polychlorinated biphenyls

Maternal and neonatal tissues were analysed for total polychlorinated biphenyls (PCBs) as commercial Aroclor mixtures 1232, 1254 and 1260. Detection limits as above.

2.5. Cord blood collection and immune responses

Cord blood samples were collected from the placental vessels into heparinized Roswell Park Memorial Institute (RPMI; Life Technology, Paisley, UK) tissue culture medium as previously described (Prescott et al., 1998). Mononuclear cells [peripheral blood mononu-

clear cells (PBMC) and cord blood mononuclear cell (CBMC)] were isolated from maternal and CB samples using Lymphoprep (Nycomed Pharma, Oslo, Norway) gradient centrifugation and cryopreserved for subsequent batch analysis. It has been shown previously that cryopreservation and thawing does not distort MC cellular immune responses (Upham et al., 2002).

For cytokine analysis, 2×10^9 CBMC and PBMC/L were cultured for 48 h in medium alone, or supplemented with optimal stimulating concentrations of house dust mite (HDM) 10 $\mu\text{g}/\text{ml}$, ovalbumin (OVA) 100 $\mu\text{g}/\text{ml}$, cat hair extract 30 $\mu\text{g}/\text{ml}$, or phytohemagglutinin (PHA) mitogen 1 $\mu\text{g}/\text{ml}$. For PHA stimulation (1 $\mu\text{g}/\text{ml}$), 10^9 CBMC and PBMC/L were used. Supernatants were assayed for the presence of interleukin (IL)-13, IL-6, IL-10 and interferon (IFN) γ employing an in-house ELISA using a time-resolved fluorometry detection system.

Lymphoproliferation responses (after 7 days in culture for CBMC/6 days for PBMC) were determined by DNA incorporation of titrated thymidine. Data were expressed as the difference in disintegrations per min (dpm) between the stimulated culture and background (unstimulated) control cultures. Responses that were greater than twice the background counts and more than 1000 dpm above the background counts were classed as positive.

2.6. Statistical analysis

Statistical analyses were carried out only on those pollutants with more than 15% detected values among the samples. These pollutants were described using the median and interquartile range (IQR). The relationship between the concentrations of pollutants and immunological parameters (i.e. cytokine protein and lymphoproliferation data) were evaluated using the Spearman rank correlation. A *P*-value less than 0.05 were considered statistically significant for all analyses. All statistical analyses were conducted with SPSS 11.0. A correction factor for multiple testing (such as a Bonferroni factor) was not applied due to the exploratory nature of this study.

3. Results

3.1. Population characteristics

Between December 2001 and June 2002 we examined 26 pairs of mothers and their healthy full term newborns. In addition, we included three samples of maternal blood at delivery for which we could not obtain corresponding infant cord blood, and two cord blood samples of newborns for which no maternal blood samples were available. The mean age of the mothers was 32.5 (range 18–41).

3.2. Levels of POPs in tissues of Western Australia women

Despite the known cumulative properties of POPs, levels in this study population have continued to fall. POPs were mainly detected in adipose tissue and breast milk, but even in these samples levels were at low levels and much lower than levels detected previously (1970s–1990s) in individuals from the same region (Stevens et al., 1993) (Table 1). POPs were not detected in the majority of cord blood and maternal blood samples (Table 2) and none of placental tissue samples showed any levels of POP contamination (data not shown).

In general, most of the individual POPs assayed were below the detectable limits of the ultrasensitive assays. Specifically, PCBs were not detectable in any of the samples in this study (data not shown; all below the minimal quantifiable level [MQL]; <0.01 mg/l). Most organochlorine (OC) compounds were also undetectable in all

samples (including endosulfan sulfate, a-endosulfan, b-endosulfan, dicofol, aldrin, lindane, heptachlor epoxide, a-chlordane, b-chlordane, *o,p'*-DDT, *p,p'*-DDD and endrin).

The most commonly detected OC compounds were *p,p'*-DDE (a metabolite of DDT pesticide), dieldrin and HCB, which were largely found in adipose samples and breast milk (Table 3). This is consistent with the well-described lipid soluble properties of these OC compounds, which can be concentrated (up to 136 times) in adipose tissues (NHMRC working party on cyclodiene insecticides, 1993). However, even in these fatty samples most other OC compounds were undetectable in this population. The most commonly detected OC in adipose (and breast milk) was *p,p'*-DDE, which was detected in 93.5% of adipose tissue samples, and 62% of breast milk samples. However, the detectable residues were at extremely low levels, far below levels that have been associated with any disease (Longnecker et al., 2001).

Table 1

Comparison of median and interquartile (IQR) of organochlorine residues in human adipose tissue^a, Western Australia, 1970–2002

Year of sampling	DDT Median (IQR)	Dieldrin Median (IQR)	HCB Median (IQR)	Heptachlor Median (IQR)	Chlordane Median (IQR)
1970 ^{b,c}	2.75	0.19	–	–	–
1988 ^d	2.40 (0.95–4.05)	0.30 (0.2–0.45)	0.85 (0.35–2.2)	ND	ND
1991 ^c	0.72 (0.16–5.20)	0.04 (0.01–1.10)	0.14 (0.01–1.07)	0.03 (0.01–0.08)	0.02 (ND–0.04)
2002 (this study)	0.07 (0.05–0.12)	0.01 (0.01–0.01)	0.01 (0.01–0.01)	0.01 (0.01–0.01)	ND ND

ND: Not detected.

^a Tissue concentration as mg/kg of extractable fat.

^b IQR not available.

^c Source; Stevens et al. (1993).

^d Source; Waddell et al. (1989).

Table 2

Median and interquartile range (IQR) of detectable organochlorine compounds in samples from WA mothers and their infants

	Maternal blood ^a (n = 29)		Umbilical cord blood ^a (n = 28)	
	Median (IQR)	N	Median (IQR)	N
Bifenthrin	0.0005 (0.0005–0.0005)	3	0.0005 (0.0005–0.0005)	7
<i>p,p'</i> -DDE	0.0005 (0.0005–0.0005)	1	–	–
b-Endosulfan	–	–	0.0005 (0.0005–0.0005)	1
HCB	0.0005 (0.0005–0.0005)	1	0.0005 (0.0005–0.0005)	1
Heptachlor	0.0005 (0.0005–0.0005)	3	0.0005 (0.0005–0.0005)	1
b-Chlordane	0.0005 (0.0005–0.0005)	1	–	–
Chlorpyrifos	0.0005 (0.0005–0.0005)	1	–	–
<i>p,p'</i> -DDT	–	–	0.0005 (0.0005–0.0005)	1
Deltamethrin	–	–	0.0005 (0.0005–0.0005)	1

N: Number of samples above the limit of detection.

^a Blood concentrations as mg/l of whole blood.

Table 3

Median and interquartile range (IQR) of detectable organochlorine compounds in adipose tissue and breast milk of WA nursing mothers, 2002

	Adipose tissue ^a (n = 31)		Breast milk ^b (n = 21)	
	Median (IQR)	N	Median (IQR)	N
p,p'-DDE	0.07 (0.05–0.12)	29	0.003 (0.001–0.009)	13
HCB	0.01 (0.01–0.01)	1	0.001 (0.001–0.001)	5
Dieldrin	0.01 (0.01–0.01)	1	0.001 (0.001–0.046)	8

N: Number of samples above the limit of detection.

^a Tissue concentrations as mg/kg of extractable fat.

^b Milk concentrations as mg/l of whole milk.

Although p,p'-DDE was more frequently detected in adipose tissue than breast milk, both HCB and dieldrin were detected more frequently in breast milk (and were rarely measurable in adipose tissue) (Table 3). As noted above, all of these residues have decreased over a period of more than 30 years (Table 1).

Although POPs can accumulate in tissues with age, in this study this phenomenon was only seen with one metabolite of DDT–1',1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) in adipose tissue, which was positively correlated with maternal age ($r = 0.40$, $p < 0.05$). There were no other relationships with maternal age.

3.3. The relationship between in vivo POP levels and (maternal and fetal) MC proliferation and cytokine responses to various stimuli

We observed a negative dose–response relationship between maternal adipose tissue levels of p,p'-DDE and maternal Type 1 (IFN γ) response to mitogen (Spearman rank correlation: $r = -0.408$, $p = 0.049$) (Table 4). Although there was a trend for other cytokine responses to be negatively correlated with p,p'-DDE levels (in particular, maternal IL-10 levels), none of these were statistically significant. No other associations other were found between POP exposure and maternal or neonatal immune parameters.

Table 4

Correlations^a between p,p'-DDE levels in adipose tissue and (maternal and fetal) MC cytokine responses to PHA mitogen (n = 26)

Cytokine	Cord blood	Maternal blood
IL-13	0.107	–0.215
IL-10	–0.201	–0.304
IFN γ	–0.144	–0.406*

$P < 0.05$ considered significant.

* $P < 0.05$.

^a Correlation coefficient (r) determined by Spearman correlation between (maternal and fetal) MC cytokines and p,p'-DDE adipose tissue levels.

4. Discussion

This novel exploratory study was intended as an important initial step in the exploration of a possible relationship between modern organic contaminants and the recent increase in allergic diseases. Because of their known biochemical properties and immunological effects, POPs represent one of a number of logical candidates in the recent environment-driven increase in allergic disease. As many as 40% of the Western Australian population show evidence of allergic sensitization (ISAAC, 1998). Moreover, in the 1980s at a time when allergic disease were rising rapidly, POPs were readily detected in adipose tissue and blood in individuals living in this region (Waddell et al., 1989). As allergic diseases have continued to increase in the intervening period, and because of the cumulative properties of these highly persistent pollutants, we recognized the need to determine if POP contamination has also increased particularly in child bearing women.

In general, we found that POP levels were in fact much lower than levels reported previously in the same population (Table 1) (Stevens et al., 1993), and most POPs were undetectable or at extremely low levels. Other studies worldwide have also noted a recent decline in tissue POP contamination (NHMRC working party on cyclodiene insecticides, 1993; Smith, 1999; Waliszewski et al., 2001). This fall in the concentration of OC pesticides is in keeping with the restrictions imposed on the use of these pesticides during the last two decades. The 19th Australian total diet survey (ANZFA, 2001) also reported a downward trend in POP concentrations in Australian foods, the major source of exposure to Western Australian adults.

We were unable to detect PCB residues at all, reducing the likelihood that these are contributing to the allergy epidemic in this region. It is still possible that PCB could have effects in other regions where they have been more readily detected in children. Extensive studies recently conducted in Germany (Waliszewski et al., 2001) and the Netherlands (Vreugdenhil et al., 2002) suggest that PCB concentrations above 400 mg/kg are associated with some adverse health effects in children

(such as change in playing behaviour and impairment of mental and motor development). The immunologic effects have not been investigated in these populations, but a previously noted association between cord blood levels of IgE and PCBs (Reichrtova et al., 1999) suggest that this is still warranted.

The falling levels of OC also reduce the likelihood that this is a major contributor to the allergy crisis in Western Australia. All subjects in this pilot study also had OC levels that fell well below any levels that have previously been associated with clinical disease (or even sub-clinical physiological effects) (Michielsen et al., 1999; Hoyer et al., 2000; Longnecker et al., 2001; Waliszewski et al., 2001; Vreugdenhil et al., 2002). Earlier studies by (Waddell et al. (1989)) found mean OC concentrations in the range of 0.1–0.35 mg/kg of adipose tissue (in WA), confirming a relative decline in these residues in the last decade. The DDT metabolite *p,p'*-DDE was detected (at very low levels) in maternal fat and breast milk in many volunteers. This is in keeping with almost universal exposure since the mid-1940s, when this compound was first used commercially. OC contamination level in human breast milk is a important measure for evaluating the toxic potential of contaminants in neonates. Although detected in over 50% of breast milk samples these were very low levels relative to global comparisons (Minh et al., 2004), indicating minimal exposure risk for these infants. Of note, DDT and its metabolites were not detected in any cord blood or placental samples. Younger women had lower levels of DDT metabolites (*p,p'*-DDE) which is in keeping with studies in other centers (Sala et al., 2001), and probably reflects reduce cumulative effects (a function of age) but also reduced environmental contamination in the recent decade.

There have been described adverse associations between neonatal outcomes and OC levels. Longnecker et al., (Longnecker et al., 2001) reported a strong association between *p,p'*-DDE levels (25 mg/l) in maternal serum and the likelihood of growth retardation and premature birth. The levels detected in the present study (in only one maternal blood sample) were vastly lower than this (0.0005 mg/l) (Table 2) reducing the likelihood of clinical effects in our population.

As for PCB, it is still possible that OC have immunological effects in regions where contamination is more significant. Even in our population, where *p,p'*-DDE was detected in most individuals but at low levels, there was a significant inverse relationship between maternal adipose tissue *p,p'*-DDE and the capacity for T helper cell Type 1 IFN γ responses (Table 4) to polyclonal stimulation (although there was no relationship with allergen responses in this population with undefined allergic status). This relationship supports our original hypothesis that the 'hormone-like' activities (Vos et al., 2000) of these compounds may favour Th2 "allergic" immune

differentiation. It also supports previous observations by Daniels et al., (Daniel et al., 2001) who demonstrated that low level OC (specifically HCB) contamination is been associated with more selectively impaired human Type 1 IFN γ responses.

Together these observations suggest that these compounds could have a differential effect on the pattern of T cell signaling. The relevance of this observation in the clinical context remains very unclear and more studies are required before any conclusions can be drawn. Neonatal Type 1 immune responses were not related to maternal *p,p'*-DDE levels, but this may because the fetus is relatively protected (below an undefined threshold) given the undetectable levels in both cord blood and placental tissues even in women with measurable *p,p'*-DDE levels.

Acknowledging that this is only a small exploratory study and may therefore lack the power to detect more subtle effects of these contaminants at the cellular level, we cannot exclude all together the role of POPs as a potential causal candidate in the increase of allergic disease, especially in developing countries where POPs are continually used for agricultural and public health purposes.

This further highlighted by a recent (2004) study in Los Angeles. Salam et al. (Salam et al., 2004) observed that asthma (an allergic disease) diagnosis before five years of age was associated with exposures in the first year of life to herbicides (OR = 4.58; 95% CI, 1.02–15.43) or pesticides (OR = 2.39; 95% CI, 1.17–4.89). In addition, an earlier study by Karmaus et al. (Karmaus et al., 2001), reported a greater than 3-fold increased risk (OR = 3.71; 95% CI, 1.10–12.56) of asthma in children between 7 and 10 years of age who had at least 0.3 μ g/l of the OC compound *p,p'*-DDE in their blood.

Finally, our PCB data were based on Aroclor measurements. This is a potential limitation of the study, which would have been more sensitive if we had measured individual PCB specific congeners, allowing for the determination and comparison of congener ratios.

In summary, our findings suggest that in POP exposure/tissue levels in Western Australian women has declined over a period when allergic disease has continued to increase. Although the relationship between DDT metabolites (*p,p'*-DDE) and reduced Type 1 capacity warrants further investigation, even women with detectable DDT/DDT metabolites, did not show significant contamination in their respective placentae or cord blood samples. This suggests that prenatal (and postnatal) exposure to POPs (at biologically relevant levels) is negligible in this randomly selected group. Thus, while there remains a plausible theoretical basis for investigating POPs as potential candidate in the development of asthma and allergies, this may not be a major contributing factor in at least the Western Australian population.

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