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REVIEW ARTICLE

Chronic adverse effects of long-term exposure of children to dichlorodiphenyltrichloroethane (DDT) through indoor residual spraying: a systematic review

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ABSTRACT

Introduction: Malaria remains a significant public health problem in endemic regions of the world, most especially in sub-Saharan Africa. As part of the global efforts to control malaria, dichlorodiphenyltrichloroethane (DDT), a cheap and effective chemical, was endorsed by the World Health Organization for use in indoor residual spraying (IRS). However, in the light of evidence on the acute toxicity of DDT, concerns have grown about the safety or the possible chronic health effects from the continued use of this persistent chemical, generating much debate and research efforts over the years. The purpose of this study was to identify, appraise and synthesise evidence about the chronic adverse effects of long-term exposure to DDT in children, 0–18 years, in zones where IRS is practised, in order to contribute to informing policy decisions.

Methods: Twenty-seven electronic databases were systematically searched using pre-defined inclusion/exclusion criteria. Two were trial registers while 25 others indexed studies of various designs. Other complementary methods were also employed in searching for both published and grey literature. Eligible studies were critically appraised using amended versions of available validated guidelines (and in a case, an improvised guideline) and a narrative synthesis was undertaken.

Results: Only nine studies met the inclusion/exclusion criteria out of 3281 hits generated. Five of the studies are of high quality while four are of moderate quality. For the three studies on neurodevelopment, evidence suggestive of negative impact of DDT was found. For the three studies on endocrine/congenital disorders, ambivalent evidence existed. In the case of the immunity-related outcome, there was growing but insufficient evidence of negative effect. The only study on survival outcome was inconclusive.

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Conclusions: Empirically, insufficient evidence exists with regard to the chronic adverse effects of long-term exposure of children to DDT through IRS. Considering the dearth of studies and the fact that many adverse effects might take much longer time to manifest, inferences drawn are weak. It would therefore require a series of well-coordinated observational studies done in the context of IRS to adequately address this evidence gap in the future.

Key words: adverse effects, Africa, DDE, DDT, dichlorodiphenyltrichloroethane, indoor residual spraying, malaria.

Introduction

A marked resurgence of malaria in the 1990s, even in countries where DDT was previously used for various purposes, led to a renewed interest in DDT use as an indoor residual spray[1,2]. While still banned or severely restricted in western countries, it was made available to the global market in 2001[3-5]. This demand for it was premised on its greater cost effectiveness and efficacy when compared with insecticide-treated bednets (ITNs) and alternatives like pyrethroids[6]. However, concerns about the safety or the chronic health effects from the continued use of this persistent chemical and its metabolite, dichlorodiphenyldichloroethylene (DDE), have generated much debate over the years[4,7-10]. Coupled with this is the seriously increasing resistance of mosquitoes to DDT[11,12] and increased likelihood of users not adhering to recommended dosage as a consequence.

Having searched the Cochrane Database of Systematic Reviews (CDSR), Database of Abstracts of Reviews of Effects (DARE) and Database of Promoting Health Effectiveness Reviews (DoPHER), we ascertained that there was no pre-existing or ongoing systematic review addressing the chronic adverse effects of long-term exposure of children less than 19 years in places where DDT is used solely or mostly for malaria control. Due to this, the ongoing debates and the unclear evidence about cumulative effects of DDT on child health[13], this review sought to identify, appraise and synthesise evidence about the chronic adverse effects of prolonged exposure to DDT on children of 0–18 years, in areas where IRS is practised, in order to contribute to informing policy decisions globally.

Methods

The protocol[14] for this review is available from the corresponding author. A widely accepted framework for standard systematic review prepared by the Centre for Reviews and Dissemination (CRD)[15] was chosen for the review design and no ethics approval was required.

Study inclusion and exclusion criteria

The PICOS (Population-Intervention-Comparator-Outcome-Study design) framework recommended by the CRD[15] was used to formulate the eligibility criteria for the review. No 'comparator' was set for this study because of the focus of the review. The framework, being flexible, was therefore modified into PIOS, which is also adequately tested[16].

As for the 'study design' component, the methodological challenge of combining results from experimental studies with observational ones was concluded to be balanced out in that the usefulness of randomised controlled trials (RCTs) as sources of adverse effects data is limited by their strict eligibility criteria (reducing generalisability), short period of follow-up, sample sizes that may not be enough to detect associations, and the fact that most of them report adverse effects as the secondary objective of study[15,17].

The final eligibility criteria set at the end of the scoping study were as follows.

Inclusion: children, 0–18 years; use of DDT in IRS for malaria vector control; outcomes being chronic adverse effects which are (i) reproductive disorders, (ii) endocrine disorders, (iii) neurodevelopmental disorders, (iv) asthma, (v) cancers, (vi) teratogenic effects (congenital abnormalities), (vii) low birthweight, (viii) immune disorders; studies published in English between1991 and 2011; RCTs, quasi-RCTs, cohort, cross-sectional and case-control studies.

Exclusion: people 19 years and older; animals; agricultural or illegal use of DDT in pest control; other malaria control measures; cost effectiveness, efficacy and anopheline resistance measures; case reports and case series.

Search strategy

The initial search strategy developed using concepts identified during the literature review, prior knowledge of abbreviations and synonyms relevant to main concepts, was piloted on EMBASE, MEDLINE and PubMed by AO to identify limitations and then reviewed by both investigators. Search terms were used for the PIO components while for the 'study design' component manual sorting was resorted to in order to avoid leaving out relevant studies. The search and study selection summary are provided in Figure 1.

Data sources

Search was conducted on 27 databases (Table 1) and the search was managed using RefWorks. The last date of search was 30 April 2011 and access was done through the University of the West of England e-library. Table 2 presents a transcript of the free text search of EMBASE (because medical subject headings (MeSH) available for a few concepts were not very inclusive). In addition, reference lists of included studies were checked to further identify relevant studies. Citations of selected articles were searched in Web of Science, PubMed, ScienceDirect and Google Scholar. World Health Organization and System for Information on Grey Literature in Europe (SIGLE) websites were also searched. All the aforementioned generated no new eligible studies. E-mails were sent to corresponding authors of included studies, attaching a copy of the inclusion/exclusion criteria for the review and requesting them to suggest any other relevant study they knew that met the criteria. The British Library was also contacted for studies whose abstracts were indicative but whose full texts were not retrievable in any of the databases searched earlier. From databases searched, 3281 hits were generated out of which 176 were found potentially relevant.

Study selection

After screening the titles and/or abstracts, 121 studies were identified from all RefWorks-compatible databases; most studies appeared in more than one database. For databases incompatible with RefWorks, selection was done onsite and 55 articles were found. Fifty three of these were found already indexed in the RefWorks-compatible databases.

Having got rid of duplicates, retrieval of the full texts of the final 46 potentially relevant articles was embarked upon by AO. Out of the six that could not to be retrieved directly, only three were later retrievable on request through the British Library. On e-mail correspondence, a reply from an author in South Africa helped in re-consideration and eventual inclusion of two previously excluded studies.

Pilot: Study eligibility criteria were piloted on all of the 40 available studies. Seven of the studies were temporarily categorised as 'eligible' and then discussed by both reviewers to verify how stringently the criteria had been applied.

Final scrutiny: After proper re-scrutiny, only nine studies were included (five selected at first, then four added on reconsideration, as indicated earlier).

For the remaining 37 that were excluded (including two from Zetoc and the two from reference-tracing) and the three that could not eventually be retrieved through the British Library, a log of the reasons for exclusion of each of them was kept for future reference[14] and is available from the corresponding author.

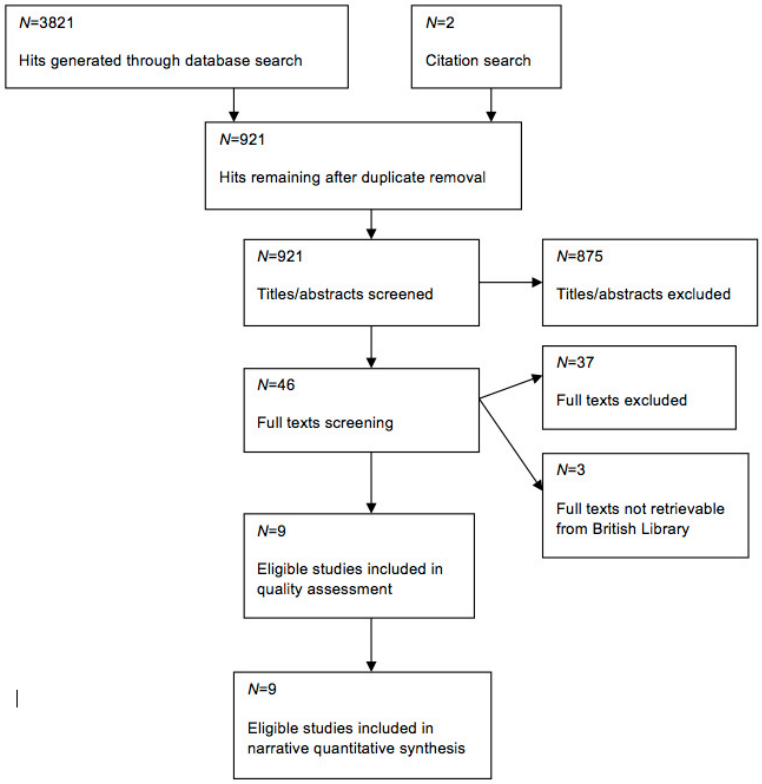

Figure 1: Study selection flow chart.

Data extraction and quality assessment

The initial data extraction form designed by us was piloted on four differently designed eligible studies. This was done alongside the quality assessment. Each data entry was done independently by AO with repeated checks to ensure correct interpretation and accuracy and thereafter moderated by DE. It enabled us to check for any necessary but missing criteria for the corresponding appraisal checklists of study types available. Our perceptions were largely similar and were resolved by consensus, where they differed.

Data were extracted on individual study characteristics including the inclusion and exclusion criteria, recruitment procedure, participant characteristics, baseline characteristics comparability, co-morbidities, number of participants enrolled, number of participants in analysis, number of withdrawals/exclusions/loss to follow-up and measurement of exposure. Data for outcome measurement and analysis included unit of assessment, outcome(s) reported, method and unit of measurement, definition of outcome, blinding of assessors, quality assurance, length of follow-up, number of times of follow-up and measurement, variables controlled for, number of participants in analysis and statistical techniques. The complete data extraction form and definitions of outcomes have been kept for future reference[14] and are available from the corresponding author.

Table 1: Databases searched

	Database
1	AMED†
2	BNI†
3	BioMedCentral (1997 to 2011)
4	CAB Abstracts (1973 to 2011 week 16)
5	CENTRAL
6	CINAHL†
7	DOAJ
8	EMBASE (1980 to 2011, week 16)
9	FreeMedicalJournals.com
10	HMIC (1979 to March 2011)
11	InformaCare†
12	InformaWorld†
13	IngentaConnect†
14	JSTOR†
15	LILACS
16	Maternity and Infant Care (1971 to April 2011)
17	MEDLINE (1948 to May 2011, week 2)
18	PubMed†
19	PsycINFO (1806 to April 2011, week 4)
20	SAGE
21	ScienceDirect†
22	SpringerLink†
23	TOXNET
24	TRoPHI
25	WileyOnline Library†
26	Web of Science (via ISI Web of Knowledge)
27	Zetoc†

† January 1991 to April 2011

Table 2: Transcript of search conducted in EMBASE

#1	(child* or adolescen* OR young OR 1-18 year*-old OR bab* OR youth* OR teenage* OR juvenile* OR pupil* OR student* OR infant*).mp.	2873335
#2	limit 1 to (human and yr="1991 - 2011")	1632026
#3	(DDT OR DDE OR organochlorine OR insectic* OR pestic* OR indoor spray* OR indoor residual spray*).mp.	78926
#4	limit 3 to (human and yr="1991 - 2011")	16457
#5	(adverse effect* OR toxicity effect* OR hazard* OR systemic effect* OR organ disorder* OR metabolic effect* OR teratogen* OR congenital abnormalit* OR chronic OR morbid* OR asthma* OR delayed psychomotor development OR poor semen quality OR cancer OR endocrine disorder* OR anti-androgen OR estrogenic effect* OR oestrogenic effect*).mp.	2981525
#6	limit 5 to (human and yr="1991 - 2011")	1930948
#7	((malaria* OR anophel* OR mosquito*) NOT (agricultur* OR net)).mp.	85913
#8	limit 7 to (human and yr="1991 - 2011")	37488
#9	2 and 4 and 6 and 8	119

Amended versions of the validated guidelines developed for the Critical Appraisal Skills Programme (CASP) of the Public Health Resource Unit, United Kingdom, were chosen to appraise cohort and case-control studies[14]. Despite extensive efforts, no validated appraisal tool was found for cross-sectional studies. Therefore, a non-validated cross-sectional study appraisal checklist obtained online from Yeshiva University[14,18] was adapted to suit the review, following a contact with an expert for guidance. Having removed irrelevant items, parameters added based on further guidance[17,19,20] included authors' knowledge base of research done in the subject area, clear definition of study sample, provision for blinding in the assessment of outcomes, cognisance of and control for potential confounders, clear statement of measures of precision and the choice of appropriate statistics and tests.

The rationale for scores apportioned *a priori* by AO to each criterion in each guideline is provided in the Results section. While not standard practice to exclude studies on grounds of quality[15], quality scores, both empirically and for specific criteria on the checklist, were used to assess the estimates of effects obtained from each study and determine their reliability.

Method of analyses and rationale

The relatively small number of eligible studies, the diversity of study designs and the methodological differences, even among the ones that examined similar outcome(s), precluded a meta-analysis[15,16], with narrative synthesis being the only possibility.

Results

From the nine studies included, four were cohort, one case-cohort and four cross-sectional. Seven of these eligible studies originated from Mexico (where DDT was used for IRS between 1991 and 2000), one from the US, although conducted for a Mexican population, and one from South Africa.

Summary of the quality assessment

Five out of the nine included studies were of high quality while four others were moderate. During assessment, greater weights were allocated to sensitive items which focused on assessment of exposure, outcome measurement, adequate control for confounders, and clarity and accuracy of data presented. The breakdown of the quality assessment scores is provided in Table 3. For the quality assessment, all study designs had a maximum attainable score of 14. The studies were graded as high (12–14); moderate: (9–11) or poor (0–8). The guidelines used and the synopses of the critical appraisal[14] are available from the corresponding author.

Results of individual studies

Summaries of the results of individual studies are given in Table 4.

Studies on adverse endocrine/congenital outcomes (*n*=3): Longnecker et al. examined the hypothesis that *in utero* exposure to DDE decreases anogenital distance (AGD, a measure of foetal androgen action or endocrine disruptor activity) in newborn males (amidst other outcomes studied)[21]. Modelling DDE concentration as a continuous variable, no association was found between quantiles of DDE concentration and any of the outcomes.

In a cross-sectional study where Torres-Sanchez et al. sampled 71 infants (37 males and 34 females) from an ongoing perinatal cohort study on neurodevelopment that started in 2001 in Morelos, Mexico, they evaluated the potential anti-androgenic effects of prenatal *p*,*p*'-DDE and *p*,*p*'-DDT exposures on infant AGD, using anal position index (API)[22]. Maternal serum levels of DDT and DDE before pregnancy and during each trimester of pregnancy were used to 'characterise' *in utero* exposure. Analysis was conducted only for *p*,*p*'-DDE because very few mothers had their *p*,*p*'-DDT levels above detection limits. A significant reduction in API (estimated difference (mm)= –0.02; 95% confidence interval (CI)= –0.03, –0.003; p=0.02) in boys was found to be associated with a doubling of maternal serum DDE levels only during the first trimester of pregnancy. No associations were found between *p*,*p*'-DDE and the age-dependent parameters (AGD and AGD/weight). For girls, no significant association was found between the parameters and DDE levels in all three trimesters.

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Table 4: Summary of study results

4a: Results from studies on impairment in infant neurodevelopment

Author (year)/ country	Initial sample size (*N*)/ design	Exposure variables (analytes measured)	Adverse outcome measures and outcome	Covariates adjusted for/ analytic technique	Results†
Eskenazi et al. (2006)/US	601 Cohort	*p,p*'-DDE *p,p*'-DDT *o,p*'-DDT	PDI, MDI Impaired neuro-development	Maternal age, education, years spent in the US, use during pregnancy, maternal depression, breastfeeding duration, maternal work status, HOME score, housing density, and poverty level, gender, age at assessment, season, location of assessment, psychometrician CSMR	**PDI and MDI models at 95%CI for infants using 10-fold increase in maternal *p,p*'-DDE, *p,p*'-DDT and *o,p*'-DDT (all in ng/g lipids). Estimated mean differences per 10-fold analyte levels (in Bayles points)** **PDI** / **MDI** **(*n*=360)** 6 months: –2.14(⁻4.20–⁻0.08)* / 0.33(⁻1.06–⁻1.73)* 12 months: –2.14(⁻4.83–0.56) / –1.15(⁻3.06–0.77) 24 months: 0.59(⁻1.58–2.77) / –2.44(⁻4.92–0.05)** **p,p'-DDT (*n*=360)** 6 months: –1.73(⁻3.36–⁻0.10)* / 0.18(⁻0.90–1.26) 12 months: –2.33(⁻4.44–⁻0.22)* / –1.71(⁻3.21–⁻0.21)* 24 months: 0.17(⁻1.54–1.88) / –2.12(⁻4.03–⁻0.21)* **o,p'-DDT (*n*=358)** 6 months: –1.47(⁻3.36–0.43) / 0.18(⁻1.06–1.42) 12 months: –1.87(⁻4.34–0.59) / –2.56(⁻4.28–⁻0.84)*** 24 months: –0.58(⁻2.61–1.44) / –3.06(⁻5.30–⁻0.83)***
Torres-Sanchez et al. (2007)/Mexico	996 Cohort	DDE (critical window of exposure)	PDI, MDI Impaired neuro-development	Birth weight, age at evaluation, breast-feeding, HOME scale at 6 months of age GMEM	**MDI and PDI models (for infants evaluated at one (*n*=225), three (*n*=233), six (*n*=227) and 12 (*n*=191) months) using doubled maternal *p,p*'-DDE (ng/mL, wet basis). Estimated mean differences (95% CI) per two-fold DDE levels** **p,p'-DDE** / **MDI** / **PDI** 1st trimester: –0.06(⁻0.36–0.24), *p*=0.69 / –0.52(⁻0.96–⁻0.075), *p*=0.02* 2nd trimester: –0.12(⁻0.43–0.20), *p*=0.47 / 0.23(⁻0.22–0.69), *p*=0.32 3rd trimester: 0.07(⁻0.24–0.40), *p*=0.64 / 0.16(⁻0.30–0.62), *p*=0.50
Torres-Sanchez et al. (2009)/Mexico	996 (carried over) Cohort	DDE (critical window of exposure)	PDI, MDI Impaired neuro development	Adjusted by maternal age, HOME scale, birth weight, breastfeeding, age at evaluation, cephalic circumference and sex of the child (and maternal IQ and height at evaluation time for MDI) GMEM	**MDI and PDI models (12, 18, 24 and 30 months) for infants using doubled maternal *p,p*'-DDE (ng/mL, wet basis); *n*=270 (those evaluated at least twice). Estimated mean differences per two-fold DDE levels** **p,p'-DDE** / **MDI** / **PDI** 1st trimester (*n*=244): 0.05(⁻0.40–0.51), *p*=0.82 / –0.08(⁻0.34–0.50), *p*=0.72 2nd trimester (*n*=153): 0.02(⁻0.50–0.53), *p*=0.95 / 0.39(⁻0.09–0.88), *p*=0.11 3rd trimester (*n*=160): 0.56(⁻0.001–1.12), *p*=0.06 / 0.37(⁻0.15–0.89), *p*=0.16

*p<0.05. **p<0.10. ***p<0.01.
CI, confidence interval. CSMR, cross-sectional multivariate regression. DDE, dichlorodiphenyldichloroethylene. DDT, dichlorodiphenyltrichloroethane. GMEM, generalised mixed effects model. HOME, home observation for measurement of the environment. MDI, mental development index. PDI, psychomotor development index.

4b: Results from studies dealing with reproductive/endocrine outcomes and preterm birth

Author (year)/ country	Initial sample size (*N*)/ design	Exposure variable (analyte measured)	Adverse measures and outcome	Covariates adjusted for/ analytic technique	Results
Longnecker et al. (2007)/ Mexico	872 (all males) Cross-sectional	DDE	AGD (1 and 2) ASD Stretched penis length Penis width Reduced androgenicity	Anogenital distances adjusted for birth weight, gestational age, urban versus rural residence, hospital, and nurse(random). Penile measurements adjusted for birth weight, maternal age, maternal height, parity, and nurse(random)	**Models for mean (95% CI) and mean differences of anthropometric parameters in boys using maternal *p,p'*-DDE concentration (µg/g of lipids); *N*=781** **DDE** **AGD1(mm)** **AGD2(mm)** **ASD(mm)** **Penis length(mm)** **Penis width(mm)** 0.1–2.9 (*n*=426) 49.9(49.1–50.7) 45.2(44.2–46.1) 18.8(18.0–19.5) 27.2(25.8–28.5) 10.7(10.4–10.9) 3.0–5.9 (*n*=175) 49.8(48.9–50.7) 45.2(44.0–46.3) 19.3(18.4–20.2) 27.1(25.7–28.6) 10.8(10.5–11.0) 6.0–8.9 (*n*=73) 49.7(48.5–50.8) 45.1(43.7–46.5) 18.5(17.3–19.6) 26.3(24.7–27.9) 10.9(10.3–10.9) 9.0–56.1 (*n*=107) 50.3(49.3–51.3) 45.6(44.4–46.9) 19.5(18.5–20.6) 27.8 (26.2–29.3) 10.9(10.6–11.2) *Estimated mean difference (mm/µg/g)* 0.029 (–0.024,0.082) –0.019 (–0.082,0.044) 0.023 (–0.028,0.074) 0.02 (–0.034,0.073) 0.010 (–0.003,0.022)
Torres-Sanchez et al. (2008)/ Mexico	83 Ongoing cohort size not given Cross-sectional	DDE	Anal position index (PA/CS or PA/CF) Anogenital distance (=PA) AGD2/weight Reduced androgenicity	Birth weight, height at birth and age at evaluation LRM	**API, AGD and AGD/weight models (95% CI) using doubled maternal *p,p'*-DDE (ng/g lipids); *n*=71** ***p,p'*-DDE (ng/g)** **Boys (*n*=37)** **Girls (*n*=34)** API 1st trimester –0.02(−0.03–−0.003), *p*=0.02* 0.003(−0.01–0.02), *p*=0.70 API 2nd trimester –0.01(−0.02–0.004), *p*=0.20 0.003(−0.01–0.02), *p*=0.82 API 3rd trimester –0.01(−0.02–0.01), *p*=0.43 –0.01(−0.03–0.002), *p*=0.09 AGD 1st trimester –0.08(−0.22–0.06), *p*=0.27 0.14(−0.05–0.30), *p*=0.35 AGD 2nd trimester –0.06(−0.20–0.07), *p*=0.36 0.11(−0.05–0.27), *p*=0.15 AGD 3rd trimester –0.12(−0.26–0.02), *p*=0.10 0.05(−0.08–0.18), *p*=0.44 AGD/weight 1st trimester –0.15(−0.37–0.06), *p*=0.16 0.18(−0.07–0.43), *p*=0.15 AGD/weight 2nd trimester –0.03(−0.23–0.17), *p*=0.75 0.09(−0.12–0.31), *p*=0.40 AGD/weight 3rd trimester –0.10(–0.31–0.11), *p*=0.33 0.08(–0.11–0.29), *p*=0.40
Bornman et al. (2009)/ South Africa	3310 Cohort	DDT-spraying status of village of residence anytime between 1995 and 2004	UGBDs detected by clinical examination: cryptorchidism (UDT), hypospadias, micropenis, chordee, phimosis, penile cyst	Maternal occupation and time lived in village MLM	**Crude data:** **Outcome** **Event rate (exposed (*n*=2396), unexposed (*n*= 914))** Cryptorchidism (UDT) 2.4%,1.3% Hypospadias 5.1%,5.4% Micropenis 2.2%,2.0% Chordee 1.6%,0.7% Phimosis 1.1%,0.9% Penile cyst 0.1%,0.4% Any UGBD 11.0%; 10.2% (incorrectly computed, actually 12.5%,10.6%) UDT (crude OR=2.1(1.14–3.92), *p* value not given): not significant Chordee (crude OR=2.5(1.1–6.0, *p*=0.04 from authors): significant **Multivariate models (*n*=3144)** Grouping all UGBDs as single variable, author obtained: **Predictor** **OR (95% CI)** Previous DDT spraying (1995–2003) 1.33(1.04–1.72), *p*=0.026* Time living in village 0.98(0.97–1.00), *p*=0.088 Homemaker 1.41(1.13–1.77), *p*=0.002* Note: Full MLM data not in original report; sent on request by authors

Torres-Arreola et al. (2003)/Mexico	233 (100 cases, 133 controls)	*p,p'*-DDE β-HCH HCB	<37 weeks of pregnancy Preterm birth	Previous preterm birth, pre-pregnancy maternal weight and prenatal care	Adjusted OR (95% CI) for preterm birth and lipid adjusted serum *p,p'*-DDE levels (ng/g) <111.6 ng/g (reference titre): *n*(cases)=23, *n*(controls)=44; OR=1.0 111.6–228.8ng/g (moderate): *n*(cases)=40, *n*(controls)=45; OR=1.87(0.95–3.68), *p* value for trend=0.17 >222.8 ng/g (high): *n*(cases)=37, *n*(controls)=44; OR=1.67(0.84–3.31)

* Statistically significant

AGD1, anogenital distance (anterior of penis to centre of anus). AGD2, anogenital distance (posterior of scrotum to centre of anus). API, anal position index. ASD, anoscrotal distance (posterior of penis to centre of anus). CF, coccyx–fourchette distance (girls). CS, coccyx–scrotum distance (boys). DDD, dichlorodiphenyldichloroethane. DDE, dichlorodiphenyldichloroethylene. DDT, dichlorodiphenyltrichloroethane. HCB, hexachlorobenzene. β-HCH, β-hexachlorocyclohexane. LRM, linear regression models. MLM, multivariate logistic regression. OR, odds ratio. PA, perineal distance. UDT, undescended testicle. UGBD, urogenital birth defects. ULR, unconditional logistic regression.

In a retrospective cohort study where Bornman et al. determined the association between six different external urogenital birth defects (UGBDs) in newborn boys and exposure to DDT from spraying in Limpopo, South Africa, both cryptorchidism and chordee were found associated with village having ever been sprayed (with wide confidence intervals)[23]. However, only chordee was significantly associated (crude odds ratio (OR)=2.5; 95% CI=1.1 to 6.0; p=0.04). Hypospadias, phimosis, micropenis and penile cyst showed no association.

In a multivariate logistic model involving 3144 out of the 3310 recruited, and where they grouped all six UGBDs as one variable (more detailed data provided on request[14]), authors claimed that the chance of male babies from villages sprayed between 1995 and 2003 having any UGBDs was 33% (OR=1.33; 95% CI=1.04 to 1.72) higher than with counterparts in villages never sprayed. Mother being a homemaker increased the risk of the child having a UGBD by 41% (OR=1.41; CI=1.13 to 1.77) compared to employed or studying mothers.

Studies on adverse neurodevelopmental outcomes (*n*=3): As part of a US multiyear birth cohort study that started in 1999/2000 with 601 mothers, a study was done by Eskenazi et al. on the effects of *in utero* exposure to two isomers of DDT (*p,p'*-DDT and *o,p'*-DDT) and *p,p'*-DDE on the neurodevelopment of infants of mothers recently immigrated from Mexico[24]. Maternal serum samples collected at the second or third trimester of pregnancy helped 'characterise' the foetal exposure having adjusted for lipid levels. The psychomotor development index (PDI) and the mental development index (MDI) of infants were measured on Bayles' scales at 6, 12 and 24 months, as indicators of neurodevelopment.

Geometric means of the serum levels of analytes (which were found to be strongly correlated, r= 0.8–0.9) were used. Statistically significant decreases of 1.73 and 2.14 points in the 6-month PDI score of infants were found to be associated with 10-fold increases in serum levels of *p,p'*-DDT (p< 0.05) and *p,p'*-DDE (p<0.05), respectively. However, only *p,p'*-DDT remained significantly associated with decreases in PDI scores (estimated difference (points)= –2.33 (95% CI= –4.44 to –0.22)) afterwards, at 12 months but not beyond. On a subgroup analysis (data not provided), authors claimed a negative association between *o,p'*-DDT level and PDI score for boys only, at 12 months (estimated difference (points)= –3.78; 95% CI= –7.47 to –0.09; p<0.01). This suggests that the non-association observed when combined was due to girls.

For MDI scores, no analyte demonstrated any association at 6 months. However, at 12 months, authors observed statistically significant decreases of 1.71 and 2.56 points in MDI scores associated with 10-fold increases in serum levels of *p,p'*-DDT (p<0.05) and *o,p'*-DDT (p< 0.01), respectively. By 24 months, 10-fold increases in serum levels of *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE were found to be associated with decreases of 2.12 (p<0.05), 3.06 (p<0.01) and 2.44 (p<0.10) points, respectively, in MDI scores. Noteworthily, a consistent negative trend, as age increased, was found with mental development.

4c: Results from studies on negative effects on immunity

Author (year)/country	Initial sample size (*N*)/design	Exposure variable (analyte measured)	Adverse outcome measures and outcome	Covariates adjusted for/analytic technique	Results
Perez-Maldonado et al. (2004)/Mexico	21 Cross-sectional	*p,p*'-DDT *p,p*'-DDE *p,p*'-DDD	Percentage of PBMCs with fragmented nuclei Apoptosis	Nil FET	**Geometric mean (SD) of percentages of apoptosis according to exposure status (to DDT spraying) on internal comparison** Unexposed (*n*=6): 0.29(0.16) Exposed (*n*=15): 2.33(2.57), $p < 0.001$ **Specific** Fischer's test on the 'exposed': DDT (p=0.06); DDE (p=0.06); DDD, (p=0.5) (DDE and DDT with marginal influence, none significant) Analysing by categorising the log-transformed concentration of each analyte into thirds, a clear trend suggestive of dose–response relationship was found with DDT and DDE on a graph.
Perez-Maldonado et al. (2006)/Mexico	2003, *N*=61 2004, *N*=57 Cross-sectional	*p,p*'-DDT *p,p*'-DDE *o,p*'-DDT	Percentage of PBMCs with fragmented nuclei Apoptosis	Age, height and weight MRM	**Regression models between mean values of percentage (log-transformed) of apoptosis and mean values of log-transformed (log base not given) DDT, DDE and DDD levels in children (6–13 years) in 2003 and 2004 at 95% CI** **Estimated difference in percentage of apoptosis** **2003 (*n*=61)** DDE: 0.38(0.17–1.24), p=0.010* DDT: 0.18(−0.26–0.98), p=0.250 DDD: 0.07(−0.07–0.18), p=0.810 Total DDT: 0.33(0.11–1.22), p=0.057 **2004 (*n*=57)** DDE: 0.36(0.24–2.55), p=0.040* DDT: 0.19(−0.74–2.19), p=0.220 DDD: 0.03(−0.07–1.39), p=0.700 Total DDT: 0.47(0.62–2.84), p=0.012* Note: Longitudinal analysis data was not provided on request

* Statistically significant
DDD, dichlorodiphenyldichloroethane. DDE, dichlorodiphenyldichloroethylene. DDT, dichlorodiphenyltrichloroethane. FET, Fischer's exact test. MRM, multiple regression models. PBMC, peripheral blood mononuclear cell. SD, standard deviation.

Among covariates adjusted for, breastfeeding was the only correlate found to be significantly positively associated with 12- and 24-month MDI scores whether DDT/DDE levels were kept in the model or not. Each month of breastfeeding was associated with increases of approximately 0.20 points in MDI scores at 12 and 24 months (p<0.05).

Torres-Sanchez et al., within a birth cohort, assessed the critical window of prenatal DDE exposure (for foetuses of mothers not occupationally exposed to DDT) and the effect it may have on PDI and MDI of infants in the first year of life[25]. To this effect, maternal serum levels of DDE before and during each trimester of pregnancy were measured and values were log-transformed to achieve normality. Adjusting for selected covariates, only with the first trimester was every doubling of serum DDE levels found to be significantly associated with a reduction in PDI scores, by 0.52 points in the first year (95% CI= –0.96 to –0.075; p=0.02). This is approximately a two-point decrease for every 10-fold increase in first-trimester maternal DDE levels. In the case of MDI, no significant association was observed with maternal DDE levels in all three trimesters.

Although a high-quality study, the very high attrition rate (approx. 75%) could have made those eventually studied different from those from the larger population. They only

demonstrated a level of comparability in very few selected baseline characteristics among those excluded, those lost to follow-up and those included in analysis. Also, no cut-offs were stated as regards the normal values for the two outcome indices.

As a follow-up to the study above, a study was done by Torres-Sanchez et al. to determine whether the effect persists between 12 and 30 months of age in a similar set of children in Morelos, Mexico[26]. Maternal serum levels of *p*,*p*'-DDE and *p*,*p*'-DDT in each trimester were evaluated on a wet basis. Only 21.2% of cohort members had *p*,*p*'-DDT levels above the detection limit and therefore not analysed. No significant association was found between DDE and either MDI or PDI scores of children at between 12 and 30 months of age, even when DDE values were adjusted for lipids, as the authors claimed (data not provided).

Compared with the earlier study, the numbers of mothers whose serum DDE levels were measured at each trimester were similar. However, the residual high attrition rate from the earlier study might have had a carryover effect. Also, the authors did not adequately account for the differences between the '333' claimed to be in the original cohort and the 244 that actually remained in this 'existing' cohort. If new members had been allowed into the cohort, findings may be affected.

Studies investigating adverse immunity-related outcomes (*n*=2): A pilot cross-sectional study was performed by Perez-Maldonado et al. examining whether DDT and its metabolites are able to induce apoptosis of human peripheral blood mononuclear cells (PBMCs) both *in vitro* and *in vivo*[27]. The levels of DDT, DDE, dichlorodiphenyldichloroethane (DDD) and the percentage of *in vivo* apoptotic cells were measured from whole blood samples of the participants, and values log-transformed to achieve normality.

For an 'internal comparison', children recruited from an unsprayed community were classified as unexposed. The geometric mean of the percentages of apoptosis was significantly higher in the exposed than in the unexposed children ($p<0.001$). Categorising children into three groups – the unexposed children being the lowest while the second and third were those with medium and high exposures respectively – a clear trend of higher apoptosis with higher exposures was found for all analytes (only graphs provided). Although without any statistical adjustments, only DDT and DDE were able to show a weak but insignificant association. However, the authors neither described their 'randomisation' nor accounted for the numbers recruited into both groups of this extremely small study but introduced them at the less rigorous analyses stage.

Two cross-sectional studies were conducted by Perez-Maldonado et al. to verify whether the blood DDT and DDE concentrations are associated with the frequency of apoptosis of PBMCs in exposed healthy children[28]. In 2003, 61 children were recruited, and, in 2004, 57 children were recruited differently; all from La Ciguena, El Ramonal and La Ventinella in Mexico. As a secondary objective, a cohort of 34 children was followed from 2002 to 2004.

Using blood samples to assess the concentrations of DDT and DDE, and the percentage of apoptosis, only DDE demonstrated a significant association with apoptosis frequencies ($p=0.10$ and $p=0.04$ for 2003 and 2004, respectively).

Study investigating adverse birth/survival outcome (*n*=1): A case-cohort study was performed by Torres-Arreola et al. in Mexico in 1995 to examine the associations that maternal serum level of *p*,*p*'-DDE and that of two other organochlorine pesticides not under review may have with preterm birth[29]. A total of 100 preterm births were recruited as cases and 133 full-term births as controls from the same cohort.

Having categorised each organochlorine into tertiles using the distribution among the controls, the adjusted ORs for preterm birth in the middle and uppermost tertiles of the *p*,*p*'-DDE concentration were 1.87 (95% CI=0.95 to 3.68, *p*(trend)=0.17) and 1.67 (95% CI=0.84 to 3.31), respectively.

Although differences in baseline risk factors were adequately adjusted for, no explanation was given for the randomisation and blinding. The relatively wide confidence intervals also suggest that the fraction of the sample size for each category of DDE concentration might have been too small to detect an association if one exists. Being a case-cohort study, only perinatal maternal serum DDE level was used, and this might not have represented the average found in cohort studies.

Comparison of studies and methodological variations detected

Broadly, no study discussed any pre-study power calculation and this was reflected in the level of robustness of many studies. All other studies except three[27-29] ensured blinding of outcome assessors to maternal exposure parameters. All studies on neonates/infants were performed on singleton births. Except for a pilot study[27], all other studies analysed effects and adjusted for confounders using regression analyses. The heterogeneity discovered across studies is indicated below.

Studies on endocrine/reproductive/congenital outcomes: A statistically significant association was only demonstrated between first-trimester maternal serum level and API in males, in Torres-Sanchez et al., where the sample size was very small (n=37) and all mothers primiparous[22]. The opposite was found with the well-powered study (a very high response rate (95%) and low attrition rate (10%)) of Longnecker et al. where most mothers had given birth previously[21]. Significant amounts of DDE excreted through previous births might have influenced outcomes here. Unlike Longnecker et al[21] however, participants' recruitment by Torres-Sanchez et al[22] might not be representative of the source population, possibly influencing the direction and magnitude of effect because infants for this cross-sectional study were recruited from an ongoing cohort. Furthermore, only one observer measured outcomes in Torres-Sanchez et al[22], and, as such, a bias might be introduced.

Bornman et al. demonstrated a significant association between a DDT-sprayed village and the occurrence of urogenital malformations, although in this study the exposure status was characterised by information in patient's file about the spraying status of their village of residence[23]. This could have predisposed the study to misclassification of participants, considering migration possibilities. There were also inadequacies in analysis and in data computed for event rates and no account was made for 166 neonates missing in their analysis, although this might not eventually alter effects sizes.

Studies on neurodevelopmental outcomes: DDE consistently demonstrated a significant association in two of these high-quality studies that investigated possible association between maternal DDE levels during pregnancy and MDI or PDI although it is noteworthy that all studies had very high attrition rates. However, in their studies Torres-Sanchez et al[25,26], unlike Eskenazi et al[24], did not adjust for whether mothers smoked during pregnancy. Although estimates of the mean for PDI and MDI scores at 6 months by Eskenazi et al[24] were similar to that of Torres-Sanchez et al[25], estimates at 12 months were much higher in the former (PDI=106±12.5 vs 91.0±7.4; MDI=100.9±9.0 vs 94.1±7.1). Differences that might account for these are that, unlike in the study of Eskenazi et al[24], where mothers had migrated out of Mexico, more of the mothers in the latter were primiparous, homemakers, much younger and still getting residual doses from the environment.

Studies on immunity-related outcomes: Out of these two successive studies conducted by Perez-Maldonado et al[27,28], only the latter, with the greater sample size, detected statistically significant association only for DDE in 2003 and 2004. The analysis in the much smaller preliminary study was less rigorous (using Fischer's test), while in the later study regression analysis was used, controlling for few confounders, though claiming that participants had similar socioeconomic backgrounds.

Discussion

Even though DDT and its metabolite, DDE, have been considered separately in the results section for clarity, both

are treated as a single putative risk factor in this section for a holistic risk assessment.

Endocrine disruptors affect the release, binding and metabolism of endogenous hormones[30,31]. Prenatal effects of these are reflected in reduced AGD (an age-dependent parameter whose clinical significance on human reproduction remains vague[30,32,33]), penile dimensions (less reliable than AGD[34]) and other developmental anomalies[35]. The two related cross-sectional studies included may have reliably showed no association between foetal DDE exposure and hormone-related anthropometric parameters in boys and girls, given that slight differences exist between serum levels at birth and during pregnancy[36]. However, Torres-Sanchez et al., with a very small sample size, demonstrated a negative association with API (an age-independent parameter calculated using AGD) in boys[22]. The main difficulty here is the limited ability to detect the temporal sequence in associations detected in cross-sectional studies[37]. There is therefore insufficient reason to conclude whether there is an association or not.

However, Bornman et al. demonstrated an association between the occurrence of any UGBD and a DDT-sprayed village[23]. A major weakness in their findings is the categorisation of all different congenital defects studied as a single variable (apparently multiple testing[19]). Such finding lacks any specificity because each birth defect may be related to other risk factors apart from DDT. Also, the retrospective nature of the study makes it vulnerable to misclassification, possibly explaining the marginal differences in event rates observed between the 'exposed' and the 'unexposed' groups (aggregates being 11.0% and 10.2% respectively). Otherwise, such a high event rate within the 'unexposed' group observed in the study should attract urgent well-planned research attention because cryptorchidism, especially, has been found to be both the most frequent abnormality of male sexual differentiation and the main risk factor for testicular cancer and male infertility[38,39].

Mild, severe or total debilitating adverse effects on the nervous system in prenatal or childhood life may not be obvious until later in life[40]. Different analyses performed to suit the various research objectives of the three studies on neurodevelopment make comparability quite challenging. However, the negative associations demonstrated between the foetal exposure to DDT/DDE and PDI scores in infants in two studies[24,25] and the consistent negative trend, as age increased, found with mental development in Eskenazi et al[24], call for deep attention. Gradual withdrawal from breastfeeding at such ages might have caused increasing expression of possible detrimental effects of DDT and its metabolites. Alternatively, the beneficial effect of breastfeeding on neurodevelopment observed in the studies (regardless of DDT/DDE exposure) might have attenuated any possible damage DDT/DDE could cause, something consistent with literature[41]. From another viewpoint, postnatal exposure could have added to the effects sizes observed because children assessed for neurodevelopmental outcomes over the course of 12–30 months would have had incremental exposure through environmental residues and breastfeeding (which increases organochlorines transfer[42]). Therefore, if the effects sizes are to be reckoned with, then they become really clinically significant at a population level, if not in individuals. Therefore, in the light of Bradford Hill criteria[37], there is a suggestive evidence on impaired mental development but no obvious causal link, as issues of specificity (although they controlled for lead), dose–response relationship and strength of association are still to be resolved.

PBMCs help in mediating inflammatory and immunological functions in the body through a release of chemical factors[43,44]. If immunosuppression should set in through much-faster-than-normal rates of apoptosis of these cells, infection susceptibility would increase. The low-precision negative associations found by Perez-Maldonado et al[28] between DDT and DDE exposure in children 6–13 years and the frequency of apoptosis of PBMCs, at different concentrations that existed in 2003 and 2004, make the evidence less reliable. There is also a difficulty in comparing this with the earlier inadequately designed study. Being cross-sectional studies, the feasibility of establishing temporal sequence between variables is dim. Also, it is difficult to

know whether DDE is the only risk factor for increased frequency of apoptosis in such populations or assess how consequential it is amidst other risk factors, thus making a concrete conclusion impossible.

Preterm birth has been identified as a major determinant of mortality and long-term morbidities like respiratory ailments, neurological impairment and developmental delay in infants although its pathogenesis has remained unclear[45]. The only included study that looked into spontaneous preterm birth[29] demonstrated no associations between sublevels of *p*,*p*'-DDE concentration in the population and preterm birth.

In a similar retrospective study in the US[46], where the median DDE level was 25µg/L (less than half than the WHO average for zones where DDT is in current use[47]), they found strong associations between the variables, following a dose–response trend. Another small case-control study[48] in Canada found no association between the variables although the median serum DDE levels were just half of that in the study under review.

Going by the contradictory evidence from the wider literature, we cannot conclude that DDE is not a risk factor for preterm birth, seeing that the study by Torres-Arreola et al[29] lacked sufficient statistical power. If there is a strong association or causal link, it should be addressed in future studies, as preterm birth implies serious consequences, especially in high-risk areas that experience very high neonatal mortality.

Of the studies included in this review, 89% were carried out on Mexican populations of varying social classes. The differences between the socioeconomic context of Mexico and that of countries most affected by exposure to DDT (from IRS), and the 'noncurrent' status of DDT spraying at the time most studies were conducted limit the generalisability of the findings. Therefore, more studies are needed from areas where DDT is currently used in IRS in order to better assess effects.

Limitations

Language bias, through the restriction of the inclusion criteria to studies written in English, was a major challenge. A relevant study found was written in Spanish, and despite extensive efforts made, no English version of it was found. This clearly indicates that a number of studies reporting in non-English-speaking countries might have been missed. Also, this English bias could have reduced the chances of indexing or citing studies reported only in their original language in many databases. Further still, some unretrieved studies could have been eligible. As well, poor technology in some parts of the world might not have enabled indexing studies performed there in electronic databases. In all, the review was based on varying levels and qualities of evidence from observational studies, with inferences that are weak for policy decisions.

Conclusions

Only very limited evidence exists about the safety of such a widely used and persistent chemical, DDT, for IRS purposes after approximately seven decades of its insecticidal use. In addition, many of the outcomes reviewed could be counted as 'mere surrogates'. Long-term, well-coordinated birth cohort studies that document outcomes until at least school age or adolescence may be required to start getting the 'true endpoints' for possible effects of DDT/DDE.

Eight out of nine included studies emanated from parts of the world where there was no 'current' use of DDT at the time of study. That, besides the methodological concerns about the types and quality of studies included, could add to explaining the weak associations found. In areas where there are sustained higher titres from current use in IRS over many years, the consequences might be more serious on children. It is therefore imperative that the WHO policy for the use of DDT for IRS be broadened to accommodate periodical evaluation of its safety in countries currently using it, with national legal frameworks monitoring the circulation and the application of DDT.

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