Chemosphere 364 (2024) 143191

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Organochlorine pesticides and their markers of exposure in serum and urine of children from a nodding syndrome hotspot in northern Uganda, east Africa

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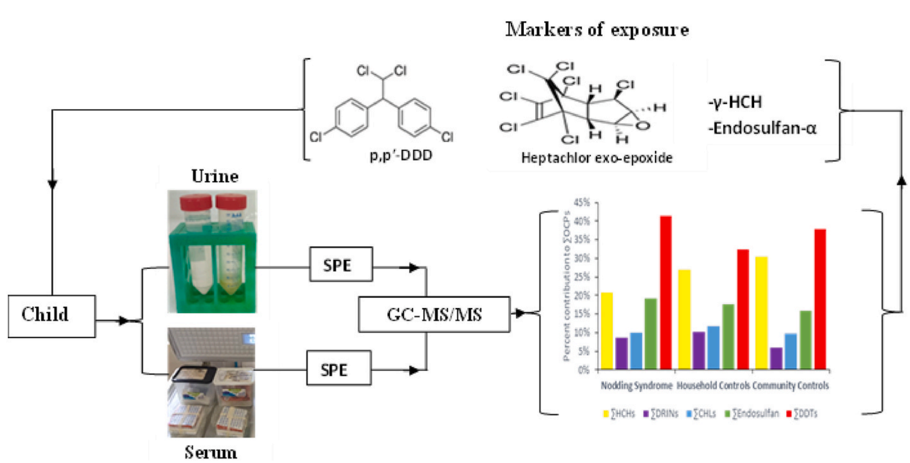
HIGHLIGHTS

- First study of persistent organochlorine pesticides (OCPs) in children from a nodding syndrome (NS) hotspot.
- OCPs in serum and urine samples were analyzed by GC-MS/MS.
- Markers of exposure in NS cases and controls were different.
- OCPs are unlikely to be associated with the etiology of NS.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: Jian-Ying Hu

Keywords:
Neurotoxic environmental chemicals
Organochlorine pesticides
DDT
Nodding syndrome
Etiology
Marker substances

ABSTRACT

Nodding syndrome (NS) is a neurologic disorder of unknown etiology characterized by vertical head nodding that has affected children aged 5–18 years in East Africa. Previous studies have examined relationships with biological agents (e.g., nematodes, measles, and fungi), but there is limited data on the possible contributions of neurotoxic environmental chemicals frequently used as pesticides/insecticides to the development and progression of this disorder. We examined the levels of persistent organochlorine pesticides (OCPs) in children (5–18 years old) from Kitgum District, Northern Uganda. These children previously lived in internally displaced people's (IDP) camps, where they were exposed to various health risks, including contaminated food and water. Exposure to OCPs through contaminated food and water is postulated here as a potential contributor to NS etiology. We analyzed serum (n = 75) and urine (n = 150) samples from children diagnosed with NS, and from

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https://doi.org/10.1016/j.chemosphere.2024.143191
Received 3 June 2024; Received in revised form 14 August 2024; Accepted 25 August 2024
Available online 29 August 2024

seizure-free household controls (HC), and community controls (CC). Samples were extracted using solid-phase extraction (SPE) and extracts were analyzed for OCPs using gas chromatography with a triple quadrupole mass spectrometry (GC-MS/MS). Mean levels of total (∑) ∑OCPs in serum samples from NS, HC and CC subjects were 23.3 ± 2.82, 21.1 ± 3.40 and 20.9 ± 4.24 ng/mL, respectively, while in urine samples were 1.86 ± 1.03, 2.83 ± 1.42, and 2.14 ± 0.94 ng/mL, respectively. Correlation and linear regression analysis indicated that potential markers for ∑hexachlorocyclohexanes (HCHs), ∑chlordane compounds (CHLs), ∑endosulfan and ∑dichlorodiphenyltrichloroethanes (DDTs) were γ-HCH, heptachlor-*exo*-epoxide, endosulfan-α and p,p'-DDD in NS cases while in controls were α –HCH, heptachlor, endosulfan-α and p,p'-DDE, respectively. Since, in some instances, higher OCP levels were found in controls vs. NS cases, we conclude that exposure to organochlorine pesticides is unlikely to be associated with the etiology of NS.

1. Introduction

Nodding syndrome (NS) is a mostly East African pediatric epileptiform encephalopathy of unknown etiology usually characterized by vertical head nodding that appears early in the course of the disease (Angues et al., 2022). New confirmed NS cases have also been reported from Central African Republic (Metanmo et al., 2021) and Cameroon (Siewe et al., 2019). Although the first descriptive reports of possible NS cases date back to the 1960s in Southern Tanzania (Mazumder et al., 2022), this form of poorly understood childhood-onset epilepsy has for past decades occurred in the conflict zones of South Sudan and Northern Uganda where community disruptions required emergency supplies of food, medicine and seeds for planting (Dowell et al., 2013; Spencer et al., 2016). The prevalence of NS varies by region, with South Sudan considered to have the highest recorded prevalence (4.6% in 2002 and 8.4% in 2013) in children aged 5–18 years (Abd-Elfarag and van Hensbroek, 2019). In 2013, the probable NS cases in Uganda were estimated at 1687 for a prevalence of 6.8 per 1000 children aged 5–18 years in the heavily impacted Districts of Kitgum, Lamwo and Pader (Spencer et al., 2016). A 2018 report by the Ugandan Ministry of Health (https://www.health.go.ug/document/statement-on-nodding-syndrome-in-northern-uganda/) indicated that there were 544 cases in Kitgum District (the location of this study) with a total of 2143 cases in Northern Uganda, and a cumulative total of 137 deaths reported between 2012 and 2018, with causes of deaths mainly arising from patients drowning in rivers and/or falling into fires during seizures, infections from severe burns, and severe malnutrition.

The cause of NS has yet to be discovered. Early research in Mundri County, South Sudan, revealed that infection with the nematodes *Onchocerca volvulus* (OV) and *Mansonella perstans* (MP) was significantly associated with NS (Tumwine et al., 2012), but subsequent study of this population showed that cases of recent (<1 year) onset were associated only with MP infection (Edridge et al., 2023). However, in Mahenge, Tanzania, established cases of epilepsy, including 38.1% of whom "met probable NS criteria," showed the presence of OV but not MP infection (Amaral et al., 2023). But OV infection is not found in the brain or cerebrospinal fluid, nor has the proposed central nervous system autoimmune reaction to a tropomysin-like extra-cerebral OV protein proved tenable (Kodja et al., 2023). Other studies have examined the associations with measles antibodies, which were positively associated with NS in Uganda (Angues et al., 2022), but the reverse was true in the South Sudanese NS cases (Tumwine et al., 2012). Since neurotropic viruses, parasites, bacteria and autoimmune factors now appear unlikely to cause NS, attention has returned to the possibility of exposure to neurotoxic environmental chemicals, whether of natural or synthetic origin (Arony et al., 2018; Spencer, 2023), including pesticides that could contaminate food and/or water used by the displaced population at high risk for NS. Moreover, previous studies have linked NS onset to exposure to toxic chemicals including munitions during the war (reviewed in Dowell et al., 2013). In addition, a potential role in NS for certain environmental biotoxins (freshwater cyanotoxins plus/minus mycotoxins) with neuroinflammatory, excitotoxic, tauopathic, and MECP2-dysregulating properties has been recently considered (Spencer et al., 2024).

Pesticides such as organochlorine pesticides (OCPs) have been extensively used in agriculture and public health for indoor residual spraying (IRS) in NS hotspot areas of Northern Uganda (Steinhardt et al., 2013; Tukei et al., 2017; Ogwang et al., 2018). For example, in Kitgum District, implementation of IRS began in 2007 and DDT was extensively applied with IDP camps being the first targets (Ogwang et al., 2018). In 2008, about 24 metric tons of DDT were used in a nationwide IRS campaign in Uganda (van den Berg et al., 2017) leading to widespread DDT contamination. DDT contamination has been reported in the atmosphere (Arinaitwe et al., 2016), soils (Ssebugere et al., 2010; Amusa et al., 2021; Mukiibi et al., 2021), sediments (Wasswa et al., 2011), and in different food sources (Kampire et al., 2011; Nannyonga et al., 2013; Mukiibi et al., 2021) from Uganda. OCPs are persistent organic pollutants (POPs) that consist of three major classes namely: dichlorodiphenylethanes, hexachlorocyclohexanes, and cyclodienes. They are characteristically ubiquitous, volatile, lipophilic, persistent and resistant to degradation (Taiwo et al., 2020). OCPs have been widely used in agriculture and public health programs leading to widespread environmental chemical contamination (van den Berg et al., 2017; Sharma et al., 2019).

OCPs can accumulate in fatty tissues of humans owing to their lipophilic and persistent nature (Waliszewski et al., 2001; Antignac et al., 2023), and they have been associated with many adverse health effects including endocrine system dysfunction, carcinogenicity, and immunotoxicity (Mrema et al., 2013). Previous epidemiological studies reported that exposure of pregnant mothers to OCPs was associated with impaired neurodevelopment and postnatal neuropsychological defects (Saravi and Dehpour, 2016). Moreover, the use of OCPs and other pesticides has been reported in populations with epilepsy (Requena et al., 2018). In India, a study by Arora et al. (2013) quantified OCPs in serum of children with idiopathic seizures. In East Africa, there are still data gaps on POPs in vulnerable populations (Ssepuya et al., 2022), including those in locations endemic to epileptic encephalopathies such as NS. In this study, we analyzed the levels of selected OCPs and their markers of exposure in serum and urine of children (5–18 years old) from a former NS hotspot area of Kitgum District, Northern Uganda.

2. Materials and methods

2.1. Study design and setting/area

This was a case-control study that recruited, where possible two age-matched controls per case. Controls were healthy children from households with NS cases and from NS-free households. The study was conducted in Tumangu Village, Kitgum District in Northern Uganda (Fig. 1). This isolated rural community is characterized by high levels of poverty, inadequate access to water, poor sanitation, and significant disease burden, and between 1987 and 2008, this region was a civil war zone (Spencer et al., 2016; Irani et al., 2019). In 1996, all civilians were moved into IDP camps where many families became dependent on emergency food supplies (Spencer et al., 2016).

2.2. Ethical consideration

This study was approved by the School of Medicine Research and Ethics Committee, Makerere University, Uganda in concert with the Oregon Health & Science University (OHSU) Institutional Review Board, Portland, USA, and it was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Parents/guardians of the donor children gave their informed consent on behalf of all child participants aged 5–18 years. Informed consent for the interview was orally administered and data was collected by the physicians who were fluent in the local Acholi language.

2.3. Study participants, sample collection and storage

The study design used here has been described in our previous publication (Angues et al., 2022). Nodding syndrome (NS) was defined according to the international consensus definition (Dowell et al., 2013). Household controls (HC) were recruited from households with NS cases, while community controls (CC) were drawn from NS-free households with no history of head nodding or any other seizures, including febrile seizures from the same community. The NS, HC, and CC subjects were randomly selected and individually screened. The demographic characteristics of the recruited participants can be found in the **Supplementary data**.

Blood samples from children were collected in vacutainer tubes and centrifuged to obtain serum (Liu et al., 2010). Serum samples were collected from NS cases (n = 50), HC (n = 50), and CC (n = 50) subjects. First-morning urine samples were collected from NS cases (n = 50), HC (n = 50), and CC (n = 50) subjects, and stored in cool boxes. Paired serum and urine samples were collected from each participant. However, half of the serum samples were shipped to OHSU, USA, and only half were analyzed in this study. All the collected samples were transported to the Pesticides Laboratory, Department of Chemistry, Makerere University, where they were frozen at −20 °C to avoid microbial decay before laboratory analysis.

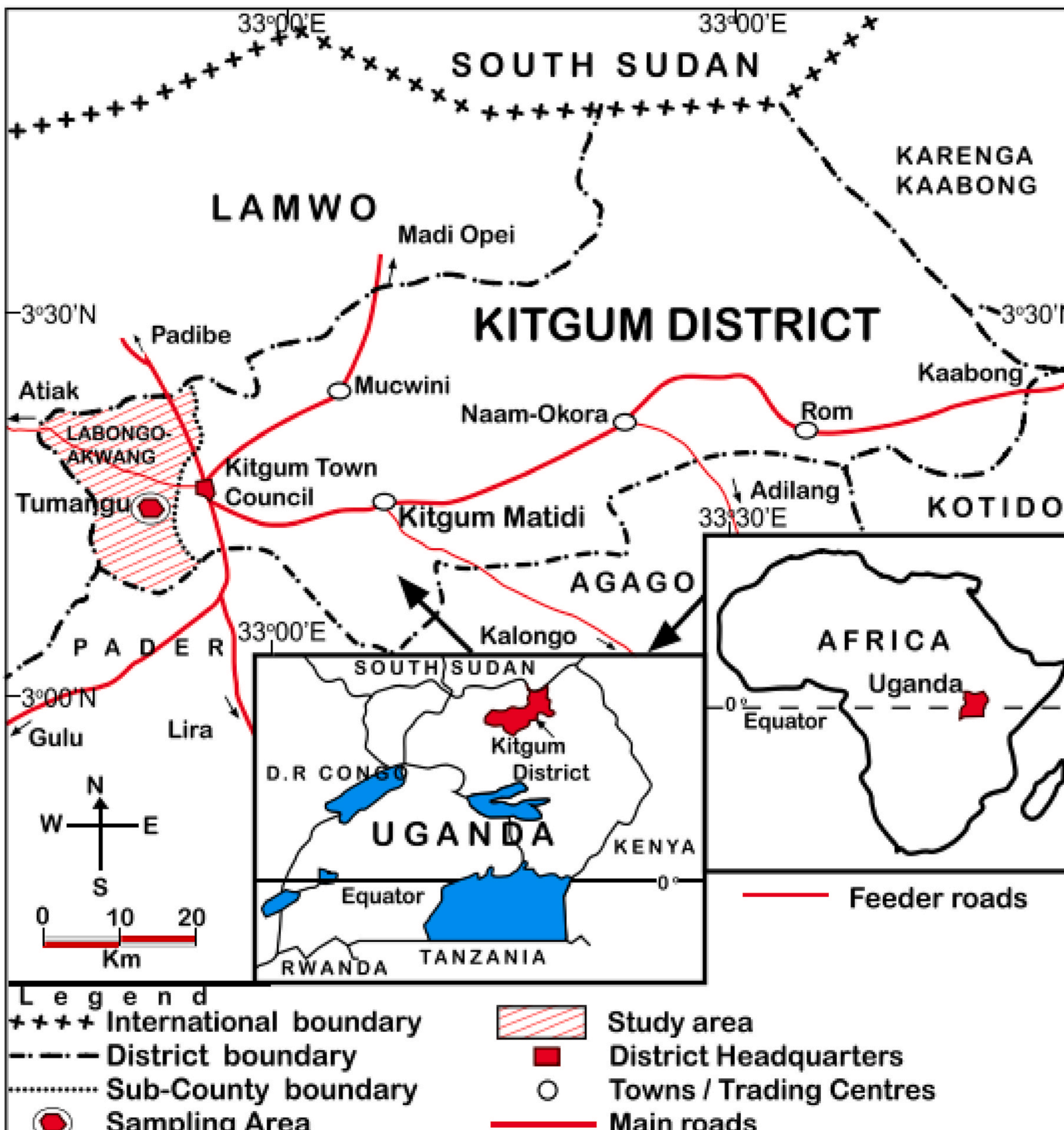

Fig. 1. The map of Kitgum District showing the sampling area (Tumangu Village).

2.3.1. Extraction of OCPs from serum samples

Serum samples were extracted following a method described by Miao et al. (2021). Thawed and homogenized serum samples (0.5 mL) were diluted with distilled water (0.5 mL) and then spiked with a mixture of surrogate standards, polychlorobiphenyl 209 congener (PCB-209) and 2, 4,5,6-tetrachloro-m-xylene (TcMX) (10 μg/L). Urea (500 mg) was then added to denature proteins in serum and the mixture was vortexed and sonicated for 30 min. The analytes were extracted from serum by solid phase extraction using Oasis® HLB extraction cartridges (3 cc/60 mg) (Waters Corporation, Milford, MA, USA) mounted on a Waters extraction manifold (Waters Corporation, Milford, USA). Before loading the samples, cartridges were conditioned with dichloromethane (DCM) (5 mL), methanol (5 mL), and then distilled water (5 mL), successively. The diluted serum samples were passed through the cartridges by gravity flow and then rinsed twice with distilled water (1 mL). The cartridges were washed with distilled water (5 mL) and vacuum-dried for 1 h by applying pressure. The analytes were then eluted using a 5 mL mixture of *n*-hexane/DCM (9:1, *v/v*). The eluate was evaporated under a gentle stream of nitrogen to near-dryness and spiked with 50 μL of pentachloronitrobenzene (PCNB) as the internal standard. The resultant mixture was reconstituted in *n*-hexane (1 mL) for gas chromatographic (GC) analysis.

2.3.2. Extraction of OCPs from urine samples

Urine samples were extracted using a method described by Cazorla-Reyes et al. (2011), but with slight modifications. A defrosted and homogenized urine sample (5 mL) was diluted with distilled water (5 mL), spiked with a mixture of surrogate standards, PCB-209 and TcMX (10 μg/L), and then vortexed. The diluted urine sample was loaded on the Oasis® HLB cartridge (6 cc/200 mg) previously conditioned with a 3 mL mixture of *n*-hexane/DCM (1:1, *v/v*) followed by ethyl acetate (3 mL), methanol (3 mL), and distilled water (3 mL). After loading the sample, the cartridge was washed with distilled water (5 mL) and vacuum-dried for 1 h by applying pressure. Afterwards, analytes were eluted using ethyl acetate (3 mL) followed by a 3 mL mixture of *n*-hexane/DCM (1:1, *v/v*). The eluate was evaporated under a gentle stream of nitrogen to near-dryness and spiked with PCNB (50 μL). The resultant mixture was then reconstituted in *n*-hexane (1 mL) for GC analysis.

2.3.3. Gas chromatographic analysis

Target analytes in the serum and urine extracts were analyzed using an Agilent Intuvo 9000 GC system interfaced with a triple quadrupole mass spectrometry (Agilent Technologies, Wilmington, Delaware, USA), using an electron impact (EI) ion source under 70 eV and operated in a multiple reaction monitoring (MRM) mode. Separation was achieved with an Ultra-Inert Intuvo GC column (DB-5MS UI; 30 m × 0.25 mm × 0.25 μm, Agilent Technologies). The following oven temperature program was used: initial oven temperature was set at 70 °C held for 2 min, increased to 200 °C at a rate of 25 °C/min, held for 10 min, and then increased at a rate of 8 °C/min to 300 °C. The column oven end-time was 41.9 min. Pure helium (99.999%) was used as a carrier gas with a constant flow rate of 1.2 mL/min. The injection port temperature was 270 °C. 1 μL of the clean extract was injected in a splitless mode with a 4 min solvent delay. The temperatures of the MS interface, ion source, and quadrupole analyzer were 300, 230, and 150 °C, respectively.

Data acquisition and processing were done using Agilent MassHunter Quantitative Analysis software. Identification of OCPs in the sample extracts was based on comparing the retention times and fragmentation patterns of the peaks in the chromatograms of the measured extracts with those in calibration standards. Six-point calibration curves were constructed and R^2 value of the standard calibration curve for each analyte was greater than 0.99.

2.4. Quality assurance and quality control (QA/QC)

The limits of detection (LODs) were calculated by a signal-to-noise ratio of three. LOD values for p,p'-DDT, p,p'-DDE, p,p'-DDD, α-HCH, β-HCH, γ-HCH, dieldrin, aldrin, endrin, endrin-aldehyde, heptachlor, heptachlor-*exo*-epoxide, endosulfan-α,-β and sulfate in serum samples varied from 0.01 to 0.13 ng/mL, while those in urine samples ranged from 0.01 to 0.11 ng/mL. Analytes were considered quantifiable when their concentrations were above LOD, while non-detects were taken to be ½ LOD (Miao et al., 2021). Recovery tests for target analytes were conducted at 5, 50, and 100 ng/mL levels, prepared by spiking QC serum and urine samples with target OCPs in triplicates. Recoveries varied from 76.5 ± 0.4% to 106.3 ± 6.3%, and 71.5 ± 1.4% to 97.7 ± 1.5% in serum and urine samples, respectively. Consequently, no recovery corrections were made since the values obtained were above 70% (Fernandes et al., 2012).

Procedural blanks were analyzed for every set of 5 samples to check for any cross-contamination. The generated OCP datasets were blank-corrected for α-HCH and heptachlor since they were found in blank. Matrix effects for the methods were determined. The detailed criteria for evaluating matrix effects used in this study have been described elsewhere (Panuwet et al., 2016; Woźniak et al., 2018). For serum samples, 9 analytes showed insignificant matrix effects (−6.9%–19.8%), 5 analytes showed distinct enhancement effects with values varying from 22.2% to 38.3%, and 1 analyte showed suppression effects (−27.1%). For urine samples, all the 15 analytes showed insignificant matrix effects with values ranging from −12.5% to 19.2%. The LODs, recoveries and matrix effects of each analyte can be found in the Supplementary data (Table S1 & Table S2).

2.5. Statistical data analysis

Statistical analysis was performed using SPSS statistic software, version 20 (IBM SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2019 software. Preliminary analysis showed that concentrations of OCPs in serum samples were normally distributed (Shapiro-Wilk test), but had unequal variances (Levene's test). As a result, a Welch's ANOVA followed by the Games-Howell post hoc test was used to evaluate the statistical differences in mean concentrations of OCPs among the different groups (NS, HC, and CC). In urine samples, concentrations of OCPs did not follow a normal distribution even after they were log-transformed. Subsequently, the statistical differences in the concentrations of OCPs among the different groups (NS, HC, and CC) were evaluated using the Kruskal-Wallis H test, followed by multiple Mann-Whitney U tests. Spearman's rank-order correlation coefficients were used to evaluate the bivariate associations between the concentrations of OCPs.

The results obtained from correlation analysis were used to identify a single OCP component that could be used as a potential marker for the groups of OCPs (∑DDTs, ∑HCHs, ∑endosulfan, ∑DRINs, and ∑CHLs) in the investigated samples. Linear regression analysis was performed between the concentrations of the potential marker substances and their corresponding groups of OCPs. The R^2 value was used to evaluate the regression since it gives an objective measure of the strength of the regression model. Subsequently, the best equations obtained from the regression analysis were used to estimate the concentrations of the groups of OCPs from their potential marker substances (Glynn et al., 2000; Wang et al., 2013). Statistical significance was considered when p-value < 0.05.

3. Results and discussion

3.1. Levels of OCP residues in serum and urine

Total (∑) OCPs in serum samples are shown in Table 1. The levels of ∑OCPs ranged from 12.8 to 30.6 ng/mL, with median and mean values of 21.7 and 21.8 ± 3.65 ng/mL, respectively. The contribution to

Table 1
Levels (ng/mL) of OCP residues in all serum and urine samples.

	All serum samples (n = 75)			All urine samples (n = 150)		
OCP residues	Mean ± SD	Median	Min - Max	Mean ± SD	Median	Min - Max
α-HCH	2.71 ± 1.58	3.09	ND – 6.90	0.29 ± 0.21	0.31	ND – 1.6
β-HCH	ND	ND	ND	ND	ND	ND
γ-HCH	2.20 ± 1.57	2.03	ND – 5.83	0.29 ± 0.41	0.35	ND – 3.88
∑HCHs	**4.92 ± 2.30**	**5.13**	**0.29–9.71**	**0.60 ± 0.49**	**0.60**	**ND – 4.66**
Aldrin	0.25 ± 0.38	0.05	ND – 1.94	ND	ND	ND – 0.74
Dieldrin	0.55 ± 1.14	ND	ND – 3.27	0.07 ± 0.12	ND	ND – 0.54
Endrin	ND	ND	ND	0.03 ± 0.08	ND	ND – 0.30
Endrin aldehyde	1.12 ± 0.70	1.47	ND – 2.45	0.10 ± 0.06	ND	ND – 0.40
∑DRINS	**1.93 ± 1.30**	**1.58**	**ND – 5.19**	**0.21 ± 0.23**	**ND**	**ND – 1.40**
Heptachlor	1.85 ± 1.14	2.69	ND – 2.82	0.11 ± 0.13	ND	ND – 0.30
Heptachlor-*exo*-epoxide	1.80 ± 1.18	2.64	ND – 2.96	0.13 ± 0.13	0.20	ND – 0.29
∑CHLs	**3.65 ± 1.53**	**3.34**	**ND – 5.75**	**0.24 ± 0.22**	**0.27**	**ND – 0.58**
Endosulfan-α	3.00 ± 1.52	3.35	ND – 5.98	0.20 ± 0.16	0.26	ND – 0.57
Endosulfan-β	1.36 ± 0.58	1.59	ND – 1.90	0.08 ± 0.08	ND	ND – 0.28
Endosulfan-sulfate	1.53 ± 0.80	1.65	ND – 5.81	0.11 ± 0.10	0.16	ND – 0.38
∑Endosulfan	**5.89 ± 1.75**	**6.53**	**1.68–10.7**	**0.40 ± 0.29**	**0.43**	**ND – 0.94**
p,p'-DDT	1.41 ± 0.72	1.61	ND – 2.71	0.11 ± 0.08	0.16	ND – 0.23
p,p'-DDE	2.03 ± 1.24	2.69	ND – 4.04	0.19 ± 0.13	0.27	ND – 0.39
p,p'-DDD	1.94 ± 1.37	1.95	ND – 6.25	0.53 ± 0.57	0.27	ND – 3.60
∑DDTs	**5.38 ± 2.14**	**5.85**	**0.59–10.9**	**0.82 ± 0.58**	**0.60**	**ND – 4.04**
∑OCPs	**21.8 ± 3.65**	**21.7**	**12.8–30.6**	**2.28 ± 1.21**	**2.20**	**0.26–7.69**

SD- Standard deviation; Min- Minimum; Max- Maximum; ND-non-detectable; α-, β-, and γ-HCH -Alpha-, beta-and gamma-hexachlorocyclohexane; DDT- Dichlorodiphenyltrichloroethane; DDE- Dichlorodiphenyl-dichloroethylene; DDD- Dichlorodiphenyldichloroethane; CHLs- Chlordanes.

∑OCPs was in the order; ∑endosulfan >∑ DDTs >∑HCHs >∑CHLs >∑DRINs. In general, the levels of ∑OCPs from our study were substantially lower than those reported in serum samples of children from industrial and pesticide storage sites, and from the major agricultural and malaria-endemic regions in Brazil, and Mexico (Freire et al., 2012; Castro-Ramirez et al., 2023), but higher than those reported from Egypt and Germany (Link et al., 2005; El Morsi et al., 2012) (Table S3).

Regarding urine samples (Table 1), levels of ∑OCPs ranged from 0.26 to 7.69 ng/mL, with median and mean levels of 2.20 and 2.28 ± 1.21 ng/mL, respectively. The contribution to ∑OCPs was in the order; ∑DDTs (36%) > ∑HCHs (26%) > ∑endosulfan (18%) > ∑CHLs (11%) > ∑DRINs (9%). There was an observable shift in the contribution to ∑OCPs from ∑endosulfan followed by ∑DDTs in serum to ∑DDTs followed by ∑HCHs in urine samples. The contributions of ∑DRINs to ∑OCPs were proportionally equal in serum and urine samples. The levels of majority OCPs found in urine samples were around LOD and at much lower levels than those in serum samples. A similar trend has been observed elsewhere (Genuis et al., 2016; Amir et al., 2021), suggesting that urine may not be a suitable matrix to reliably measure the body burdens and elimination pathways of more lipophilic compounds such as OCPs. Moreover, a previous study by Amir et al. (2021) did not find any correlation between urine and serum levels of DDT metabolites. Majority of OCPs have an octanol-water partition coefficient, $\log K_{OW} > 5$ (Shen and Wania, 2005), implying that they are highly lipophilic and fat soluble, and are thus sequestered in fatty tissues. These chemicals are barely transferred to aqueous biofluids such as urine which influences their partitioning between serum and urine as observed in studies elsewhere (Genuis et al., 2016; Amir et al., 2021; Hardy et al., 2021). To date, there is limited literature about the levels of OCPs in urine from children, with only a few studies reported in the adult population (Table S4).

3.1.1. DDTs

The levels of ∑DDTs in all serum samples varied from 0.59 to 10.9 ng/mL, with mean and median levels of 5.38 ± 2.14 and 5.85 ng/mL, respectively (Table 1). Composition analysis of ∑DDTs showed that p,p'-DDE (38%) was the dominant component followed by p,p'-DDD (36%), and p,p'-DDT (26%). The mean levels of p,p'-DDE, p,p'-DDD, and p,p'-DDT in all serum samples were 2.03 ± 1.24, 1.94 ± 1.37, and 1.41 ± 0.72 ng/mL, respectively. The observed trend in the composition of ∑DDTs found in this study was comparable to that reported by Meza-Montenegro et al. (2013) who found mean DDT levels in serum samples of Mexican children in the order: p,p'-DDE >p,p'-DDD >p,p'-DDT. Studies have shown that the accumulation potential of OCPs in more advanced species is greatly influenced by the metabolic properties of the chemical, where p,p'-DDE has been observed to have a higher bio-accumulation potential in human tissues compared to other DDT metabolites resulting in higher p,p'-DDE concentrations (Zumbado et al., 2005; Al-Saleh et al., 2012; Xu et al., 2017).

Furthermore, biological transformation of technical grade DDT, which contains about 75% p,p'-DDT to p,p'-DDE, in addition to ingestion of previously degraded p,p'-DDT, has been shown to increase p,p'-DDE levels in human tissues (Xu et al., 2017). According to the literature, p,p'-DDE/p,p'-DDT, and (DDE + DDD)/∑DDTs ratios in serum can be used to indicate recent and past input of DDT, where (DDE + DDD)/∑DDTs ratio greater than 0.5 indicates past exposure, otherwise, a recent/fresh exposure is suggested (Xu et al., 2017; Yin et al., 2020). In this study, the overall mean (DDE + DDD)/∑DDTs ratio was 0.74, with 89% of serum samples showing values greater than 0.5, and the more predominant p,p'-DDE/p,p'-DDT ratio >1 suggested that past use of DDT was the most likely source of DDT contamination in the study area. The level of ∑DDTs found in serum samples from this study was generally higher than those reported in children from Germany (Link et al., 2005; Bandow et al., 2020), Northwestern Mexico (Meza-Montenegro et al., 2013), and Egypt (El Morsi et al., 2012), but lower than those from Brazil (Freire et al., 2012) and Central Mexico (Castro-Ramirez et al., 2023) (Table S3).

Regarding urine samples, the level of ∑DDTs ranged from non-detectable (ND) to 4.04 ng/mL, with a mean of 0.82 ± 0.58 ng/mL (Table 1). Composition analysis of ∑DDTs indicated that p,p'-DDD (64%) was the dominant component, followed by p,p'-DDE (23%), and p,p'-DDT (13%). The level of p,p'-DDT varied from ND to 0.23 ng/mL, with a mean of 0.11 ± 0.08 ng/mL while p,p'-DDE ranged from ND to 0.39 ng/mL (0.19 ± 0.13 ng/mL), and p,p'-DDD ranged from ND to 3.6 ng/mL (0.53 ± 0.57 ng/mL). The overall mean of (DDE + DDD)/∑DDTs ratio was 0.88, with 100% of the positive samples showing values higher than 0.5, and p,p'-DDE/p,p'-DDT ratio was greater than 1, which suggested past use of DDT in the study area.

3.1.2. Endosulfan

The Level of ∑endosulfan in all serum samples ranged from 1.68 to 10.7 ng/mL with a mean level of 5.89 ± 1.75 ng/mL (Table 1). The mean of ∑endosulfan found in this study was higher than that reported in children from Mexico (Meza-Montenegro et al., 2013), but lower than that reported from Brazil (Freire et al., 2012). The levels of endosulfan-α ranged from ND to 5.98 ng/mL, while endosulfan-β and endosulfan-sulfate varied from ND up to 1.90 and 5.81 ng/mL, respectively. It could be observed that the serum endosulfan-sulfate levels were higher than endosulfan-β, similar to a trend observed in Pakistan and Spain (Carreño et al., 2007; Attaullah et al., 2019). Moreover, the maximum serum endosulfan-sulfate levels reported by Carreño et al. (2007) were up to eight times higher than endosulfan-β, unlike in our study where serum endosulfan-sulfate levels were about three times higher than endosulfan-β.

In urine samples, the levels of ∑endosulfan varied from ND to 0.94 ng/mL with a mean of 0.40 ± 0.29 ng/mL. Among the endosulfan isomers, endosulfan-α (51%) was the predominant component, followed by endosulfan-sulfate (28%), and endosulfan-β (21%). The levels of endosulfan-α, endosulfan-sulfate, and endosulfan-β varied from ND up to 0.57 (0.2 ± 0.16 ng/mL), 0.38 (0.11 ± 0.1 ng/mL) and 0.28 (0.08 ± 0.08 ng/mL), respectively. Endosulfan-sulfate is a main metabolite of endosulfan (α- and β-isomers), it degrades slower and is more abundant in food, environmental media, and human matrices worldwide (Sathishkumar et al., 2021). Endosulfan has been used worldwide to eradicate pests from crops, especially in vegetables, fruits, cotton, cereals, and tobacco. It was listed for elimination under the Stockholm Convention in 2011, though it is still being used in a few countries, including China and India (Yan et al., 2021). In Uganda, endosulfan contamination has been reported in vegetables and other food sources including honey and cow milk (Kampire et al., 2011; Nannyonga et al., 2013; Mukiibi et al., 2021).

3.1.3. HCHs, DRINs and CHLs

As shown in Table 1, the levels of ∑HCHs in all serum samples ranged from 0.29 to 9.71 ng/mL, with a mean of 4.92 ± 2.3 ng/mL. Levels of ∑DRINs varied from ND up to 5.19 (1.93 ± 1.3 ng/mL), whilst ∑CHLs were up to 5.75 (3.65 ± 1.53 ng/mL). ∑HCHs + DRINs + CHLs contributed 48% to ∑OCPs in serum. Among the HCHs, β-HCH was below LOD in all samples while α-HCH and γ-HCH (lindane) had mean levels of 2.71 ± 1.58 and 2.2 ± 1.57 ng/mL, respectively in serum samples, and 0.29 ± 0.21 and 0.29 ± 0.41 ng/mL, respectively in urine. In 2009, α-HCH, β-HCH, and lindane were listed in the Stockholm Convention as POPs, and efforts have since been made to restrict their production and use worldwide (Ramos et al., 2011; Vijgen et al., 2011, 2019, 2022). Technical HCH is a mixture of five HCH isomers, usually in the proportions of; 55–80% (alpha-HCH), 5–14% (beta-HCH), 8–15% (gamma-HCH), 2–16% (delta-HCH) and 3–5% (epsilon-HCH). Lindane constitutes more than 90% gamma-HCH (Vijgen et al., 2022).

Globally, lindane pollution in the environment is well documented, and studies have reported that about 4.8–7.2 million tons of HCH wastes (especially HCH isomers) have been generated from the production of about 600,000 tons of lindane (Vijgen et al., 2011, 2019). Former HCH production sites, storage sites, and stockpiles continue to serve as conduits of lindane pollution (Vijgen et al., 2019, 2022). In Uganda, lindane contamination has been reported in soil and honey sampled near an abandoned pesticide store (Mukiibi et al., 2021). Dietary intake is the major pathway for lindane exposure in humans, accounting for more than 99% (Sandu and Virsta, 2015). Previous studies (Ogwok et al., 2009; Kampire et al., 2011; Nannyonga et al., 2013) have reported lindane contamination in the different food sources in Uganda. Similarly, lindane and other HCH isomers have been reported in serum/plasma samples of children and adult populations worldwide (Freire et al., 2012; Saoudi et al., 2014; Bandow et al., 2020; Miao et al., 2022; Castro-Ramirez et al., 2023). The mean level of ∑HCHs in serum samples from our study was higher than that reported in children from Germany (Bandow et al., 2020), Mexico (Meza-Montenegro et al., 2013), and Egypt (El Morsi et al., 2012), but lower than that reported in children from Brazil (Freire et al., 2012).

Among the DRINs, the contribution to ∑DRINs in all the serum samples was in the order: endrin-aldehyde (58%) > dieldrin (29%) > aldrin (13%) > endrin (0%). The levels of endrin-aldehyde, dieldrin, and aldrin were up to 2.45, 3.27 and 1.94 ng/mL, respectively. In urine samples, the levels of ∑DRINs ranged from ND to 1.4 ng/mL with a mean of 0.21 ± 0.23 ng/mL, and it is worth noting that endrin-aldehyde and dieldrin all combined accounted for 96% of the ∑DRINs composition. The more predominant endrin-aldehyde and dieldrin were quantified with mean levels of 0.1 ± 0.06 and 0.07 ± 0.12 ng/mL, respectively. In the biota, aldrin is rapidly transformed to dieldrin, whilst endrin, having a short half-life (about 24 h in blood) is rapidly metabolized/biodegraded in blood (Zitko, 2003), which could explain why traces of endrin were not found in the investigated serum samples. The levels of ∑DRINs found in this study were lower than those reported in the serum samples of children from Brazil (Freire et al., 2012).

Regarding CHLs, the mean levels of heptachlor and heptachlor-*exo*-epoxide in all serum samples were 1.85 ± 1.14 and 1.8 ± 1.18 ng/mL, respectively. The serum mean level of ∑CHLs in this study was lower than that reported in children from Brazil (Freire et al., 2012). In urine samples, the mean level of ∑CHLs was 0.24 ± 0.22 ng/mL. Among the CHLs, heptachlor-*exo*-epoxide was quantifiable in 52% of the urine samples with a mean level of 0.13 ± 0.13 ng/mL, while heptachlor was quantifiable in 38% of the samples with a mean of 0.11 ± 0.13 ng/mL.

3.1.4. Nodding syndrome cases versus controls

The levels of ∑OCPs in serum samples from NS, HC and CC subjects ranged from 17.2 to 30.6, 14.4 to 29.7, and 12.8–27.2 ng/mL, respectively (Table 2). The mean levels of ∑OCPs among NS, HC, and CC were statistically significantly different ($p< 0.05$, Welch's ANOVA test). The post-hoc test showed statistically significant differences between the means of ∑OCPs for NS cases vs. HC ($p< 0.05$, Games-Howell). On the contrary, the mean levels of ∑OCPs for NS cases vs. CC, and HC vs. CC were not statistically significantly different ($p> 0.05$, Games-Howell). The observed difference in OCPs levels among the different groups could be attributed to variations in age, body mass index, body weight, and sex of children (Bandow et al., 2020). The mean levels of OCPs in the serum samples were in the order; ∑DDTs >∑endosulfan >∑HCHs >∑CHLs >∑DRINs in NS cases; ∑DDTs >∑endosulfan >∑HCHs > ∑CHLs >∑DRINs in HC subjects; and ∑endosulfan >∑HCHs > ∑DDTs >∑CHLs > ∑DRINs in the CC subjects. The mean level of ∑DDTs was statistically significantly different among the different groups ($p< 0.05$). Among the DDTs, composition analysis revealed that p,p'-DDT had the lowest contribution to ∑DDTs, and in all cases, the proportion of ∑(p,p'-DDE + p,p'-DDD) was about three times higher than p,p'-DDT. Among the DRINs, the more persistent dieldrin had the highest mean level in NS cases (0.77 ± 1.24 ng/mL), followed by CC (0.63 ± 1.26 ng/mL), and then HC (0.26 ± 0.88 ng/mL). The mean levels of endrin-aldehyde followed a similar trend. Among the endosulfan isomers, endosulfan-α had the highest level in all cases (Table 2).

In urine samples, the levels of ∑OCPs in NS, HC and CC subjects ranged from 0.26 up to 6.15, 7.69, and 4.48 ng/mL, respectively (Table 3). The levels of ∑OCPs among HC, CC and NS were statistically significantly different ($p< 0.05$, Kruskal-Wallis H test), but in NS cases vs. HC ($p< 0.05$, Mann-Whitney U test). The contribution to ∑OCPs was in the order; ∑DDTs > ∑HCHs > ∑endosulfan >∑CHLs > ∑DRINs. The levels of ∑DDTs from NS, HC and CC were up to 4.04, 2.32, and 1.74 ng/mL, respectively. p,p'-DDD had the highest contribution to ∑DDTs, and in all cases, the proportion of ∑(p,p'-DDE + p,p'-DDD) > p,p'-DDT. The levels of ∑HCHs from NS, HC and CC were up to 0.77, 4.66, and 1.63 ng/mL, respectively. The levels of ∑endosulfan in NS, HC, and CC ranged from ND to 0.89, 0.80, and 0.94 ng/mL, respectively, while that of ∑CHLs varied from ND to 0.54, 0.58, and 0.55 ng/mL, respectively. The levels of ∑DRINs ranged from ND up to 0.48, 1.40, and 0.77

Table 2
Levels (ng/mL) of OCPs in serum samples from nodding syndrome cases and controls.

	Community Controls (n = 25)		Household Controls (n = 25)		Nodding Syndrome (n = 25)	
OCP residues	Mean ± SD	Min - Max	Mean ± SD	Min - Max	Mean ± SD	Min - Max
α-HCH	2.35 ± 1.55	ND – 5.80	3.16 ± 1.96	ND – 6.90	2.61 ± 1.08	ND – 3.18
β-HCH	ND	ND	ND	ND	ND	ND
γ-HCH	2.12 ± 1.75	ND – 5.83	1.69 ± 1.41	ND – 4.43	2.78 ± 1.37	ND – 4.36
∑HCHs	**4.49 ± 2.17**	**1.17–7.50**	**4.86 ± 2.85**	**0.29–9.71**	**5.41 ± 1.74**	**2.05–7.55**
Aldrin	0.45 ± 0.47	ND – 1.94	0.24 ± 0.34	ND – 1.31	0.06 ± 0.16	ND – 0.74
Dieldrin	0.63 ± 1.26	ND – 3.20	0.26 ± 0.88	ND – 3.27	0.77 ± 1.24	ND – 3.09
Endrin	ND	ND	ND	ND	ND	ND
Endrin aldehyde	1.22 ± 0.78	ND – 2.45	0.88 ± 0.71	ND – 2.14	1.26 ± 0.54	ND – 1.62
∑DRINS	**2.31 ± 1.30**	**ND – 5.19**	**1.38 ± 1.17**	**ND – 4.55**	**2.10 ± 1.27**	**ND – 4.66**
Heptachlor	1.59 ± 1.32	ND – 2.82	1.80 ± 0.94	ND – 2.71	2.16 ± 1.10	ND – 2.77
Heptachlor-*exo*-epoxide	1.97 ± 1.17	ND – 2.96	1.41 ± 1.16	ND – 2.86	2.02 ± 1.16	ND – 2.87
∑CHLs	**3.56 ± 1.61**	**ND – 5.75**	**3.21 ± 1.50**	**ND – 5.34**	**4.19 ± 1.36**	**2.65–5.64**
Endosulfan-α	3.18 ± 1.77	ND – 5.98	3.04 ± 1.61	ND – 5.10	2.78 ± 1.12	ND – 3.66
Endosulfan-β	1.40 ± 0.58	ND – 1.90	1.15 ± 0.73	ND – 1.66	1.54 ± 0.32	ND – 1.66
Endosulfan-sulfate	1.68 ± 1.03	ND – 5.81	1.52 ± 0.71	ND – 2.47	1.38 ± 0.61	ND – 1.74
∑Endosulfan	**6.26 ± 2.13**	**1.84–10.7**	**5.72 ± 1.78**	**1.68–7.70**	**5.70 ± 1.25**	**3.11–6.90**
p,p'-DDT	1.15 ± 0.81	ND – 2.71	1.53 ± 0.78	ND – 2.65	1.55 ± 0.48	ND – 2.16
p,p'-DDE	1.46 ± 1.30	ND – 4.04	2.38 ± 1.08	ND – 3.72	2.25 ± 1.18	ND – 3.35
p,p'-DDD	1.68 ± 1.51	ND – 4.58	2.03 ± 1.08	ND – 3.52	2.11 ± 1.48	ND – 6.25
∑DDTs	**4.29 ± 2.43**	**0.59–8.25**	**5.94 ± 1.59**	**2.19–8.37**	**5.91 ± 1.94**	**2.59–10.9**
∑OCPs	**20.9 ± 4.24**	**12.8–27.2**	**21.1 ± 3.40**	**14.4–29.7**	**23.3 ± 2.82**	**17.2–30.6**

Table 3
Levels (ng/mL) of OCPs in urine samples from nodding syndrome cases and controls.

	Community Controls (n = 50)		Household Controls (n = 50)		Nodding Syndrome (n = 50)	
OCP residues	Mean ± SD	Min - Max	Mean ± SD	Min - Max	Mean ± SD	Min - Max
α-HCH	0.41 ± 0.26	ND – 1.60	0.30 ± 0.12	ND – 0.76	0.17 ± 0.15	ND – 0.40
β-HCH	ND	ND	ND	ND	ND	ND
γ-HCH	0.22 ± 0.25	ND – 0.75	0.45 ± 0.60	ND – 3.88	0.20 ± 0.20	ND – 0.42
∑HCHs	**0.65 ± 0.30**	**ND – 1.63**	**0.76 ± 0.68**	**ND – 4.66**	**0.39 ± 0.29**	**ND – 0.77**
Aldrin	0.01 ± 0.01	ND – 0.11	0.04 ± 0.11	ND – 0.74	0.01 ± 0.01	ND – 0.1
Dieldrin	0.02 ± 0.06	ND – 0.35	0.11 ± 0.15	ND – 0.54	0.06 ± 0.12	ND – 0.31
Endrin	ND	ND	0.08 ± 0.12	ND – 0.30	ND	ND
Endrin aldehyde	0.09 ± 0.07	ND – 0.40	0.12 ± 0.05	ND – 0.19	0.08 ± 0.05	ND – 0.16
∑DRINS	**0.13 ± 0.12**	**ND – 0.77**	**0.35 ± 0.31**	**ND – 1.40**	**0.16 ± 0.14**	**ND – 0.48**
Heptachlor	0.11 ± 0.13	ND – 0.28	0.15 ± 0.13	ND – 0.30	0.06 ± 0.11	ND – 0.27
Heptachlor-*exo*-epoxide	0.10 ± 0.13	ND – 0.28	0.18 ± 0.11	ND – 0.29	0.12 ± 0.13	ND – 0.27
∑CHLs	**0.21 ± 0.24**	**ND – 0.55**	**0.33 ± 0.20**	**ND – 0.58**	**0.19 ± 0.17**	**ND – 0.54**
Endosulfan-α	0.17 ± 0.18	ND – 0.57	0.26 ± 0.15	ND – 0.43	0.18 ± 0.16	ND – 0.42
Endosulfan-β	0.07 ± 0.08	ND – 0.22	0.12 ± 0.07	ND – 0.26	0.07 ± 0.07	ND – 0.17
Endosulfan-sulfate	0.10 ± 0.12	ND – 0.38	0.13 ± 0.09	ND – 0.26	0.10 ± 0.09	ND – 0.34
∑Endosulfan	**0.34 ± 0.36**	**ND – 0.94**	**0.50 ± 0.22**	**ND – 0.80**	**0.36 ± 0.26**	**ND – 0.89**
p,p'-DDT	0.07 ± 0.08	ND – 0.19	0.12 ± 0.07	ND – 0.23	0.14 ± 0.06	ND – 0.20
p,p'-DDE	0.13 ± 0.15	ND – 0.35	0.22 ± 0.11	ND – 0.39	0.22 ± 0.10	ND – 0.35
p,p'-DDD	0.61 ± 0.58	ND – 1.73	0.55 ± 0.54	ND – 1.89	0.41 ± 0.58	ND – 3.60
∑DDTs	**0.81 ± 0.49**	**ND – 1.74**	**0.89 ± 0.61**	**ND – 2.32**	**0.77 ± 0.63**	**ND – 4.04**
∑OCPs	**2.14 ± 0.94**	**0.26–4.48**	**2.83 ± 1.42**	**0.26–7.69**	**1.86 ± 1.03**	**0.26–6.15**

ng/mL for NS, HC and CC, respectively. Levels of OCPs were generally greater in serum compared to urine samples, which could imply low urinary elimination of OCPs in children.

3.2. Marker substances for OCPs exposure

Serum levels of the individual organochlorines and their corresponding sums; ∑OCPs, ∑DDTs, ∑DRINs, ∑endosulfan, ∑HCHs, and ∑CHLs were positively correlated, with Spearman's rank order correlation coefficient (r) ranging from 0.101 to 0.898 in all cases. Positive correlations showed that certain individual components could be used as marker substances for the groups of OCPs and only statistically significant correlations ($p< 0.05$) among the individual organochlorine were considered (Glynn et al., 2000). Conversely, the data of OCPs from our urinary analysis did not satisfy the aforementioned statistical criteria and were thus excluded from further analysis. In serum samples from the NS cases, the greatest statistically significant correlations for ∑DDTs, ∑HCHs, ∑endosulfan, ∑CHLs, and ∑DRINs were p,p'-DDD ($r = 0.818$, $p< 0.01$), γ-HCH ($r = 0.815$, $p< 0.01$), endosulfan-α ($r = 0.808$, $p< 0.01$), heptachlor-*exo*-epoxide ($r = 0.710$, $p< 0.01$), and dieldrin ($r = 0.512$, $p< 0.01$), respectively. Weaker correlations were observed between p,p'-DDE and ∑DDTs ($r = 0.386$, $p> 0.05$), aldrin and ∑DRINs ($r = 0.217$, $p> 0.05$), and between endosulfan-β and ∑endosulfan ($r = 0.145$, $p> 0.05$).

In serum samples from HC, the greatest statistically significant correlations for ∑DDTs, ∑HCHs, ∑endosulfan, ∑CHLs, and ∑DRINs were p,p'-DDE ($r = 0.642$, $p< 0.01$), α-HCH ($r = 0.898$, $p< 0.01$), endosulfan-α ($r = 0.693$, $p< 0.01$), heptachlor ($r = 0.760$, $p< 0.01$), and endrin-aldehyde ($r = 0.573$, $p< 0.01$), respectively. Weaker correlations were observed between p,p'-DDT and ∑DDTs ($r = 0.419$, $p< 0.05$), endosulfan-sulfate and ∑endosulfan ($r = 0.101$, $p> 0.05$), heptachlor-*exo*-epoxide and ∑CHLs ($r = 0.479$, $p< 0.05$), aldrin and ∑DRINs ($r = 0.407$, $p< 0.05$), and dieldrin and ∑DRINs ($r = 0.470$, $p< 0.05$). From the CC serum samples, the greatest statistically significant correlations for ∑DDTs, ∑HCHs, ∑endosulfan, ∑CHLs and ∑DRINs were p,p'-DDE ($r = 0.780$, $p< 0.01$), α-HCH ($r = 0.696$, $p< 0.01$), endosulfan-α ($r =$

0.892, $p < 0.01$), heptachlor ($r = 0.735$, $p < 0.01$) and dieldrin ($r = 0.564$, $p < 0.01$), respectively. Conversely, weaker correlations were found between p,p'-DDT and $\sum$DDTs ($r = 0.477$, $p < 0.05$) as well as between endosulfan-sulfate and $\sum$endosulfan ($r = 0.434$, $p < 0.05$), endosulfan-β and $\sum$endosulfan ($r = 0.210$, $p > 0.05$), heptachlor-*exo*-epoxide and $\sum$CHLs ($r = 0.436$, $p < 0.05$), aldrin and $\sum$DRINs ($r = 0.137$, $p > 0.05$) and between endrin-aldehyde and $\sum$DRINs ($r = 0.438$, $p < 0.05$).

Linear regression was performed in the cases where correlation coefficients were greater than 0.60 (Wang et al., 2013). Correlation analysis indicated that in NS cases, heptachlor-*exo*-epoxide might be a good marker substance for $\sum$CHLs ($R^2 = 0.902$), while endosulfan-α ($R^2 = 0.894$), p,p'-DDD, ($R^2 = 0.838$) and γ-HCH ($R^2 = 0.866$) might be potentially good markers for $\sum$endosulfan, $\sum$DDTs and $\sum$HCHs, respectively. For HC subjects, correlation analysis indicated that heptachlor might be a good marker for $\sum$CHLs ($R^2 = 0.800$), while endosulfan-α ($R^2 = 0.915$), p,p'-DDE ($R^2 = 0.807$) and α-HCH ($R^2 = 0.932$) might be good marker substances for $\sum$endosulfan, $\sum$DDTs, and $\sum$HCHs, respectively. Similarly, for CC subjects, correlation analysis indicated that heptachlor ($R^2 = 0.812$) and endosulfan-α ($R^2 = 0.910$) might be good markers for $\sum$CHLs and $\sum$endosulfan, respectively, but p,p'-DDE and α-HCH showed $R^2 < 0.75$.

From our study, it could be observed that there was a clear shift in marker substances for $\sum$CHLs from heptachlor-*exo*-epoxide in NS cases to heptachlor in controls; $\sum$HCHs from γ-HCH in NS cases to α-HCH in controls, and for $\sum$DDTs from p,p'-DDD in NS cases to p,p'-DDE in controls, but future research with bigger sample sizes are needed to confirm our findings. A study by Glynn et al. (2000) demonstrated that inclusion of age in the regression model did not significantly improve R^2 value. From our study, there were no good markers for $\sum$DRINs due to small detection rates of the individual DRINs, and their small regression coefficients. A similar observation was previously reported by Wang et al. (2013). The levels (Table S5) of the $\sum$HCHs, $\sum$endosulfan, $\sum$CHLs, and $\sum$DDTs were estimated from their corresponding markers using equations from the regression analysis.

Furthermore, the difference between estimated and measured concentrations for $\sum$HCHs, $\sum$CHLs, $\sum$endosulfan and $\sum$DDTs was calculated using equation (1) (Wang et al., 2013).

$$\textit{Percent difference} = \left(\frac{\textit{Estimated concentration}}{\textit{Measured concentration}} - 1\right) \times 100 \tag{1}$$

The percentage differences between estimated and measured concentrations of $\sum$HCHs, $\sum$CHLs, $\sum$endosulfan and $\sum$DDTs are shown in Fig. 2. From this study, a difference of between −10% and 10% was observed in 82%, 77%, and 74% of the serum samples corresponding to NS, HC and CC for all the organochlorines. In no case was the observed difference greater than 38% where the NS cases showed a maximum difference of 19%, while HC and CC subjects showed maximum differences of 23% and 38%, respectively.

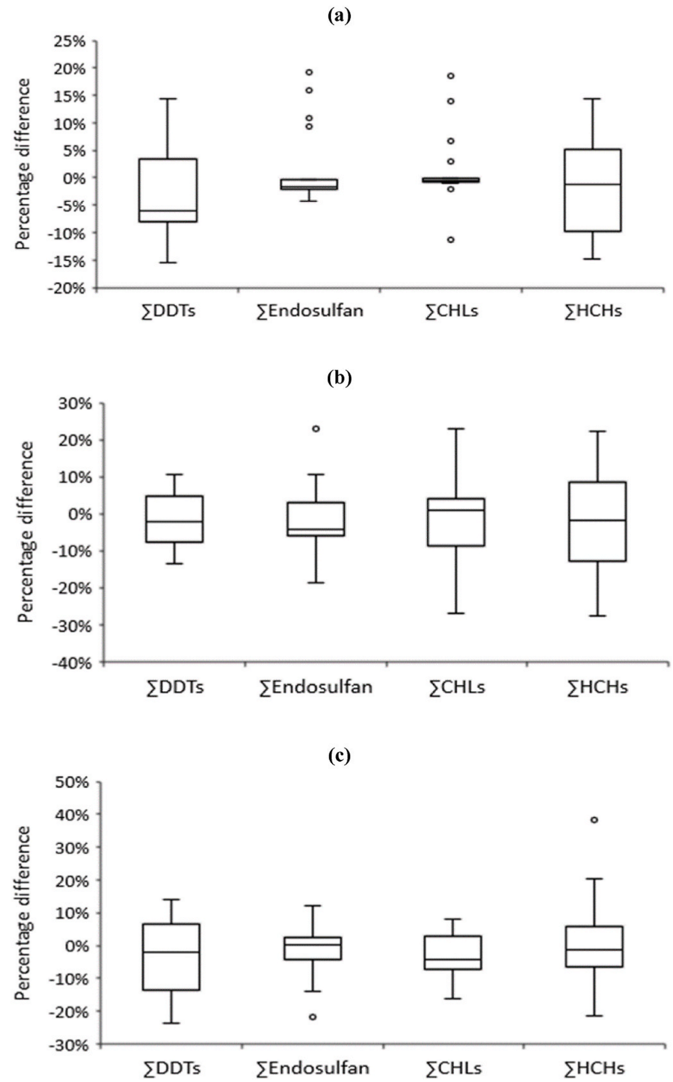

Fig. 2. Box and whisker plots for the percentage difference between estimated and measured serum OCPs concentrations from: (a) NS cases, (b) HC subjects, and (c) CC subjects.

4. Conclusions

This study investigated the occurrence of OCPs in serum and urine of children from a former NS hotspot in Northern Uganda. Levels of $\sum$OCPs in all serum samples ranged from 12.8 to 30.6 ng/mL, and contribution to $\sum$OCPs was in the order: $\sum$endosulfan > $\sum$DDTs > $\sum$HCHs > $\sum$CHLs > $\sum$DRINs. The levels of $\sum$OCPs in serum samples from NS, HC, and CC subjects ranged from 17.2 to 30.6, 14.4 to 29.7, and 12.8–27.2 ng/mL, respectively. In all urine samples, $\sum$OCPs ranged from 0.26 to 7.69 ng/mL, and contribution to $\sum$OCPs was in the order; $\sum$DDTs > $\sum$HCHs > $\sum$endosulfan > $\sum$CHLs > $\sum$DRINs. Levels of $\sum$OCPs in urine samples from NS, HC, and CC ranged from 0.26 up to 6.15, 7.69, and 4.48 ng/mL, respectively.

Furthermore, our results from correlation and linear regression analysis indicated that potential markers for $\sum$HCHs, $\sum$CHLs, $\sum$endosulfan and $\sum$DDTs were γ-HCH, heptachlor-*exo*-epoxide, endosulfan-α and p,p'-DDD in NS cases while in controls were α-HCH, heptachlor, endosulfan-α and p,p'-DDE, respectively. Organochlorine pesticides were detected in NS cases and controls and, in some instances, controls presented higher OCP levels than NS cases. While samples were collected from children with NS years after disease onset, the analysis focused on chemical contaminants that persist for years or decades (Olisah et al., 2020). Our study results suggested that NS could not be linked to OCP exposure, but future studies with bigger sample sizes from other NS areas are needed to confirm our findings.

Funding

This work was supported by the National Institutes of Health (grant no: 3R21NS108355–01S1 REVISED; subaward no: 1013078-004_MAKERERE); Third World Academy of Sciences (grant no. 20-267 RG/CHE/AF/AC_G), International Science Program (grant no: UGA 01) and APPEAR Academic Partnership (Project 249-ECSDevelop).

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Resources, Methodology, Investigation, Supervision, Writing–Review & Editing. **Peter S. Spencer:** Funding acquisition, Conceptualization, Study Design, Sampling, Supervision, Writing–Review & Editing. **Valerie S. Palmer:** Funding acquisition, Conceptualization, Study Design, Sampling, Writing– Review & Editing. **Raquel Valdes Angues:** Funding acquisition, Conceptualization, Study Design, Sampling, Writing– Review & Editing. **Amos Deogratius Mwaka:** Study Design, Sampling, Review & Editing. **John Wasswa:** Funding acquisition, Resources, Conceptualization, Study Design, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors thank all the participants, Tumangu Village Health Team Leader, and the medical team for their invaluable contribution to this study. We also thank Steven Mulinda for his technical assistance during laboratory analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143191.

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