LONG-TERM NEURODEVELOPMENTAL EFFECTS OF ACUTE ORGANOPHOSPHATE POISONING AMONGST SOUTH AFRICAN CHILDREN

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Abbreviations

ADHD	Attention deficit/ hyperactivity disorder
ASEBA	Achenbach System of Empirically Based Assessment
CBCL	Child behaviour checklist
DSM	Diagnostic and Statistics Manual
ICU	Intensive care unit
NPH	Nonpreferred hand
OP	Organophosphates
OPIDP	Organophosphate induced delayed polyneuropathy
OPP	Organophosphate poisoning
OR	Odds ratio
PSS	Poison severity score
PH	Preferred hand
RXH	Red Cross Hospital
SA	South Africa
SDG	Sustainable development goals
SSA	Sub-Saharan Africa
TROG	Test for the Reception of Grammar
VMI	Visual motor integration

Abstract

Background: Pesticide poisoning is a significant cause of morbidity and mortality amongst children in developing countries. In addition to the well described acute effects, organophosphates(OP), can cause long-term neurotoxicity. This study aims to evaluate long-term neurodevelopmental outcomes in a sample of paediatric survivors of acute organophosphate poisoning (OPP) a subject which has, to date not been well described.

Objectives: This study determines the performance of South African children surviving acute OPP on a validated set of paediatric neurodevelopmental tests. It compares the performance of acute OPP survivors to two control groups matched for age, sex, and home language.

Methods: A case-control study was conducted. A group of OPP survivors (cases) was compared to two control groups: (1) children admitted for paraffin poisoning; and (2) children admitted for conditions other than poisoning. Participants were identified through hospital records. Consenting participants were interviewed and evaluated using six neurodevelopmental tests. 47 cases of acute OPP were recruited and matched to 46 cases of paraffin poisoning and 29 non-poisoned controls.

Results: In the comparison of the OPP and control groups, The OPP group performed significantly worse in grooved pegboard, fingertap repetition, total problem score and anxious/depressed clinical syndrome scale. In the comparison of the OPP and paraffin groups, the OPP group performed significantly worse for grooved pegboard, total problem score, social problems clinical syndrome scale, attention deficit/hyperactivity disorder (ADHD) and conduct diagnostic and statistics manual (DSM) scales.

Conclusion: This study suggests that OPP in children can result in neurodevelopmental deficits across a range of domains: motor functioning and speed, behavioural problems, attention and emotional wellbeing. These impacts appear to be specific to OP and not just the result of hypoxia associated with poisoning. It highlights the need for more effective poisoning prevention measures, and long-term follow up, neurodevelopmental assessment and support of OP-poisoned children into adolescence.

Key words: organophosphates, pesticides, children, neurotoxicity, neurodevelopment

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Publication ready manuscript

Author guidelines for the Environmental Health Perspectives journal are contained in Appendix 5.

Long-term neurodevelopmental effects of acute organophosphate poisoning amongst South African children

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Introduction

Poisoning is a significant cause of morbidity and mortality amongst children particularly in developing countries. In 2019, there were over 77 000 reported deaths from unintentional poisonings globally. One quarter of these occurred in sub-Saharan Africa (SSA), with children being disproportionately affected. In the same year, children under 5 years in SSA accounted for 60% of all deaths due to unintentional poisoning.[1]

In South Africa (SA), pesticide poisoning has been reported as the most common non-drug chemical exposure accounting for 20% of all poison centre consultations, and 9% of all poisoning admissions in one hospital study in the Western Cape.[2] Of these, cholinesterase inhibitors were the most commonly reported pesticide exposure.[2,3] Additionally, acute poisonings are a key reason for admission to hospital and particularly intensive care units (ICU).[2,4,5]

Highly hazardous agricultural pesticides are increasingly used in household settings in SA for pest control, particularly in impoverished areas where overcrowding and poor sanitation often results in pest infestation. These so called "street pesticides" made from agricultural pesticides are sold cheaply and illegally to residents in low-income communities, posing significant risk particularly to children.[6,7] Children's behaviour, interaction with their environment as well as their rapidly developing brain make them particularly vulnerable to adverse effects following exposure to neurotoxic pesticides.[8-11]

Organophosphates (OP), cholinesterase inhibitors, are one class of commonly used pesticides.[11,12] They are highly toxic and were amongst the most widely used until the 21st century. Many have been banned or their use heavily regulated in many high-income countries due to their toxicity. In addition to the well described acute cholinergic syndrome, OPs can cause an intermediate syndrome, organophosphate-induced delayed polyneuropathy (OPIDP), as well as long-term neurotoxicity.[11-17]

In children, there have been several studies examining the consequences of pre-natal exposure in utero, or low-levels early childhood exposures to organophosphate and organochlorine pesticides, which have suggested deficits in attention, executive function, short-term memory, impaired motor coordination and visual performance, increased reaction time, impaired mental development, impaired neonatal reflexes, pervasive emotional or developmental difficulties and even structural brain anomalies.[9,10,18-24]

However, the literature describing the long-term effects of acute poisonings in children is not as extensive, particularly compared to that existing for adults. There are case reports which have shown late-onset distal polyneuropathies in 2 children,[25] impairments in balance, reaction time, colour vision, trails making and grooved pegboard test in 3 children,[26] as well as 9 children with subsequent difficulties with both motor inhibition and verbal learning memory tasks.[27] The latter study focused on functions related to the forebrain nuclei. Neuro-anatomically, the basal ganglia comprise a cholinergic rich brain area that has also been demonstrated to be sensitive to in the acute phase to organophosphate neurotoxicity.[28]

This study therefore provides a unique opportunity to evaluate long-term neurodevelopmental outcomes in a sample of paediatric survivors of acute OPP which has to date not been well described. It aims to

compare performance of acute OPP survivors to two control groups on a standard set of neurodevelopmental tests. This will also enable better delineation of the full impact of acute pesticide poisoning as well as the long term needs of survivors.

Methods

This was a prospective case-control study conducted at Red Cross Children's Hospital (RXH), which is a tertiary paediatric hospital in Cape Town, SA.

Study population

A group of OPP survivors (cases) was compared to two control groups: (1) children admitted for poisoning where the agent was not a pesticide (paraffin); and (2) children admitted for conditions other than poisoning. All participants were admitted to RXH between 1 January 2001 and 31 December 2011, aged between 3 and 12 years at the time of enrolment and first language English, Afrikaans, or isiXhosa.

Index cases were children admitted to RXH with a diagnosis of acute OPP during the period. A diagnosis of acute OPP was based one or more of the following criteria: (1) a documented physician diagnosis of acute OPP based on clinical sign and symptoms; (2) positive red blood cell cholinesterase level; and or (3) documented response to atropine or pralidoxime. Cases with any previous history of acute or chronic organophosphate poisoning were excluded.

Cases of OPP and paraffin poisoning were identified through the hospital's poison's centre database. Non-poisoned controls were identified from hospital records. Controls were matched to cases 1:1 on date of admission (within 6 months of admission), age (within 6 months), gender and first language.

Children were excluded from the study if they met any of the following criteria: (1) any pre-existing conditions known to be associated with a neurodevelopmental problem or disability: (2) documented or reported history of an acute neurologically-impairing event (including, but not limited to meningitis, head trauma with intracranial bleed or diffuse axonal injury); (3) history of epilepsy; (4) known mental health conditions; (5) any child known to be HIV positive on records (no active HIV testing was undertaken); (6) any underlying medical, neurologic or behavioural condition that might influence performance in neurodevelopmental testing (including, but not limited to, cerebral palsy, autistic spectrum disorder).

Our sample size calculation aimed to recruit 50 index cases matched 1:1 with paraffin-poisoned and non-poisoned controls. Due to lower response rate among non-poisoned controls, we were not able to match index cases to controls as initially anticipated. A total of 47 cases of acute OPP were recruited and matched to 46 cases of paraffin poisoning and 29 non-poisoned controls. See appendix 1 for further details on methodology contained in study protocol.

Data collection

Once potential study participants were identified through hospital records, their parent or primary caregiver were contacted by a member of the research team and invited to attend a clinic at RXH to be

provided with more detailed study information. After providing informed consent and assent (for children over 7 years) we obtained information on participants' sociodemographic, and medical history.

Each participant was then interviewed and tested by a trained test administrator in their primary language. The assessors were recruited from staff involved in previous studies at the RXH Child Guidance Clinic and had been thoroughly trained in assessment methods for the various tools. Test administrators were blinded to participants' exposure group (OPP, paraffin, or control). Testing was conducted at one point in time during the recruitment period (1 October 2012 – 31 July 2013).

Ethical approval for this study was provided by the University of Cape Town Human Research and Ethics Committee (HREC 468/2007).

Neurodevelopmental assessment

Neurodevelopmental tests were chosen based on anticipated domains likely to be affected by OP insult.[20,29-31] Each patient was evaluated using six standardised tests. The time required to complete the entire battery of tests was between one to two hours. After assessment, a report summarising the test battery results was prepared for each child's primary caregiver and the results explained to them.

Ravens Colored Progressive Matrices

This cross-cultural test assesses non-verbal reasoning assessment. It is based on matching components of patterns and is normed for children aged 3 years 6 months to 11 years. It has been used widely in cognitive research in developing countries.[32-34] It is suited for children 3 to 14 years old.[35]

Test for the Reception of Grammar (TROG)

This test measures verbal capacity and understanding of grammatical contrasts. It consists of identification of 80 items depicting increasing complexity of syntactic and lexical grammar.[36]

Both the Ravens and TROG tests have been successfully used to evaluate cognitive function in the South African population in previous studies as proxies for more extensive IQ tests.[32-34,37-39] These tests were appropriate for the nature of this study; they are less time and labour intensive and could be conducted effectively by a trained individual without a psychometry or psychology background.

Beery Developmental Test of Visual Motor Integration (VMI)

This test assesses visual-motor integration by requiring the child to copy 24 geometric figures. It is constructed to measure the level of visual-motor development from 3 to 14 years of age. It has been used extensively in research studies and provides a sensitive measure of fine motor problems in children.[37,40,41] The overall VMI score is a good predictor of reading ability.[42]

Digit span

This is a test of attention and working memory, which are components of the wider domain of executive function. Executive functioning is influenced by the basal ganglia, which may be impaired in children with OPP. The test has a forward and backward phase. The tester first reads a sequence of numbers

forwards which the child repeats in the same manner. If no errors are made the span is increased until two sets of responses at the same span are incorrect. The child is then instructed to repeat a sequence numbers, but backwards and the span is again increased until 2 consecutive errors are made at the same span. This test shows good reliability and validity and is commonly included in many IQ batteries. For children aged 3 to less than 8 years, the digit span test from the Junior South African Individual Scales (J-SAIS) was used. For children aged 8 to 17 years, the same test from the Senior South African Individual Scales-Revised was used.

Grooved pegboard test

The test assesses fine motor dexterity and coordination. It consists of a pegboard with 25 grooved slots. The child is required to place a grooved peg into each of the slots first with the right hand and then the left hand. The time to complete the task with each hand is recorded. This is useful in the context of OPP to elicit fine motor and hand speed deficit suggestive of extra-pyramidal tract problems, known to occur in chronic OPP.[40,43-46]

Finger tapping

This test assesses the integrity of the neuromuscular system and examines motor control and dexterity.[40,44,46] The child is asked to tap a key for a series of 10 -15 seconds, first with the preferred hand (PH) then the non-preferred hand (NPH). The average sum of taps from the non-practice session with each hand is recorded.

Achenbach System of Empirically Based Assessment (ASEBA) Child Behavior Checklist questionnaire (CBCL)

These scales make up a comprehensive evaluation of childhood behaviour and social functioning. These are useful to capture behavioural characteristics particularly those related to attention and concentration. The CBCL measures a child's competence and problem areas as perceived by the parent or caregiver. There are two versions: one for children 1.5 to 5 years with 100 questionnaire items, and another for 6 to 18 years of age with 113 items. The CBCL provides raw scores, T-scores and percentiles for: 3 summary, 8 clinical syndrome, 7 DSM-IV-oriented and 3 competence scales. This would be useful in detecting any reported attentional deficits in children with OPP relative to controls.[47]

Data analysis

Statistical analyses were performed using STATA version 17.0 (Statacorp, College Station, Texas). Demographics (age at time of testing, gender, race, socioeconomic status), and outcome measures (Raven, TROG, Beery-Buktenica Test, Digit Span, Grooved Pegboard Test, Finger tapping and Achenbach CBCL Scales) were compared between the control group and the children in the paraffin and acute OPP groups separately. Nominal data were analyzed with χ^2 statistics. For data that were not normally distributed, the nonparametric Wilcoxon's rank sum test was used. For normally distributed data, Student's t-test was used. Tests were conducted at a 5% level of significance.

Neurodevelopmental outcomes that were noted to be significantly different in the bivariate analyses of OPP vs controls, and paraffin vs controls were included in the logistic regression.

Logistic regression was used to explore the association between neurodevelopmental outcomes and exposure to acute OPP and paraffin. This analysis was restricted to cases with complete data. Given the small sample size, for generation of binary outcomes, neurodevelopmental test outcomes were converted to binary outcomes based on quartiles. For each outcome variable, raw scores for the entire group were grouped into quartiles, with the highest quartile (Q4) compared to quartiles 1-3. The models were adjusted for age, sex and socioeconomic status. In the comparison of the OPP and paraffin groups the models were also adjusted for poisoning severity as measured by poison severity score (PSS). The PSS is a classification scheme that was used to asses severity of acute poisonings at initial presentation.[48] Measures of effect were structured to indicate poorer performance on neurodevelopmental outcomes as a higher odds ratio. Significance was set at 0.05, with 95% confidence intervals reported. Although we intended to carry out a matched design, we were not able to achieve sufficient numbers to do so, due to the low response rate in the non-poisoned controls.

Results

A total of 122 children were included in the study. Table 1 presents the maternal and socio-demographic characteristics of study participants.

Table 1: Socio-demographic characteristics of study participants

	Control N=29	Paraffin N=46	OPP N=47	Total N=122	p-value
Sex					**0.298**
Male	15 (51.7)	32 (69.6)	29 (61.7)	76 (62.3)	
Female	14 (48.3)	14 (30.4)	18 (38.3)	46 (37.7)	
Age (years)					**0.765**
Median (IQR)	7.5 (6.6-8.5)	7.4 (6.1–10.4)	7.9 (6.3–10.4)	7.5 (6.3–9.8)	
School grade					**0.741**
Preschool	8 (27.6)	15 (32.6)	11 (23.4)	34 (27.9)	
Grade 1-3	15 (51.7)	18 (39.1)	22 (46.8)	55 (45.1)	
Grade 4-7	6 (20.7)	13 (28.3)	14 (29.8)	33 (27.0)	
Testing language					**0.436**
Afrikaans	2 (6.9)	2 (4.4)	6 (12.8)	10 (8.2)	
English	4 (13.8)	3 (6.5)	8 (17.0)	15 (12.3)	
Xhosa	22 (75.9)	39 (84.8)	33 (70.2)	94 (77.1)	
English/ Xhosa/ Afrikaans	1 (3.4)	2 (4.4)	0 (0.0)	3 (2.4)	
Maternal variables					
Age (years)†					**0.463**
Median (IQR)	35 (31-38)	33 (29-39)	36 (30-40)	34 (30-50)	
Primary caregiver/ parent employed					**0.005**
Yes	18 (62.1)	17 (37.0)	18 (38.3)	53 (43.4)	
No	11 (37.9)	29 (63.0)	23 (48.9)	63 (51.6)	
Unknown	0 (0.0)	0 (0.0)	6 (12.8)	6 (4.9)	
Education level					**<0.001**
Primary	2 (6.9)	3 (6.5)	3 (6.4)	8 (6.6)	
Secondary	27 (93.1)	43 (93.5)	32 (68.1)	102 (83.6)	
Unknown	0 (0.0)	0 (0.0)	12 (25.5)	12 (9.8)	
No of dependants					**0.003**
0 – 3	21 (72.4)	34 (73.9)	30 (63.8)	85 (69.7)	
4 – 7	8 (27.6)	12 (26.1)	8 (17.0)	28 (22.9)	
Unknown	0 (0.0)	0 (0.0)	9 (19.2)	9 (7.4)	
Type of housing					**0.001**
Brick house	19 (65.5)	24 (52.2)	24 (51.1)	67 (54.9)	
Shack	9 (31.0)	22 (47.8)	12 (25.5)	43 (35.3)	
Unknown	1 (3.5)	0 (0.0)	11 (23.4)	12 (9.8)	
Composite SES score‡					**0.674**
Quartile 1	7 (24.1)	10 (21.7)	14 (29.8)	31 (25.4)	

Quartile 2	8 (27.6)	18 (39.1)	12 (25.5)	38 (31.1)	
Quartile 3	8 (27.6)	12 (26.1)	7 (14.9)	27 (22.1)	
Quartile 4	6 (20.7)	6 (13.0)	6 (12.8)	18 (14.8)	
Missing	0 (0.0)	0 (0.0)	8 (17.0)	8 (6.6)	
Marital status					**0.082**
Married or cohabiting	17 (58.6)	15 (32.6)	19 (40.4)	51 (41.8)	
Other¥	12 (41.4)	31 (67.4)	28 (59.6)	71 (58.2)	

†15 participants with missing maternal age

‡Composite SES score comprised of Z scores from 4 variables: employment status, type of housing, maternal education level and number of dependents.

¥Includes single parent, separated/ widowed and 6 unknown cases.

The median age at study enrolment was 7.5 years. The first language of the majority (77.1%) of participants was isiXhosa. The median maternal age was 34 years. There was a higher proportion of mothers who were employed in the control group (62.1%) compared to those in the paraffin (37.0%) and acute OPP (38.3%) poisoning groups. Overall, 83.6% of mothers had completed secondary level education. This proportion was slightly lower in the acute OPP group (68.1%).

Table 2 presents the characteristics of participants exposed to paraffin and organophosphates on initial presentation. The median age at exposure (initial presentation) was significantly higher in the acute OPP compared to the paraffin group (2.4 vs 1.5 years). A higher proportion of participants in the acute OPP group were hospitalised (93.6% versus 73.9%) and admitted to ICU (29.8% versus 8.7%) compared to paraffin-poisoned controls. Participants in the acute OPP group also had significantly higher PSS, and more than one third (34.0%) had a PSS of 3.

Table 2: Characteristics of participants exposed to paraffin and organophosphates

	Paraffin Control N=46	OPP N=47	Total N=93	p-value
Age at exposure (years)‡				**<0.001**
Median (IQR)	1.5 (1.2-1.6)	2.4 (1.5-4.2)	1.6 (1.3-3.0)	
Time between exposure & testing (years)				**0.293**
Median (IQR)	6.6 (5.7-9.2)	7.3 (5.9-9.7)	6.8 (5.8-9.4)	
Route of exposure				**0.368**
Ingestion	32 (69.6)	39 (83.0)	71 (76.3)	
Inhalation	0 (0.0)	1 (2.1)	1 (1.1)	
Unknown	14 (30.4)	7 (14.9)	21 (22.6)	
Poison severity score (PSS)				**<0.001**
1	26 (57.8)	9 (19.1)	35 (38.0)	
2	12 (26.7)	13 (27.7)	25 (27.2)	
3	6 (13.3)	16 (34.0)	22 (23.9)	
2 or 3	0 (0.0)	8 (17.0)	8 (8.7)	
Unknown	1 (2.2)	1 (2.1)	2 (2.2)	
Hospitalised				**<0.001**
Yes	34 (73.9)	44 (93.6)	78 (83.9)	
No	12 (26.1)	3 (6.4)	15 (16.1)	
Days hospitalised	**N=34**	**N=44**		**0.006**
Median (IQR)	2 (1-2)	3 (2-4)	2 (2-3)	
ICU				**<0.001**
Yes	4 (8.7)	14 (29.8)	18 (19.3)	
No	30 (65.2)	32 (68.1)	62 (66.7)	
Unknown	12 (26.1)	1 (2.1)	13 (14.0)	
Days in ICU				**0.149**
Median (IQR)	4 (1.5-8)	2 (1-2)	2 (1-2)	
ICU mortality score				**0.705**
Median (IQR)	0.139 (0.014-0.159)	0.033 (0.021-0.119)	0.037 (0.021-0.139)	
Composite severity score†				**0.002**
1	19 (67.9)	8 (25.8)	27 (45.8)	
2	6 (21.4)	5 (16.1)	11 (18.6)	
3	1 (3.6)	9 (29.0)	10 (16.9)	
4	2 (7.1)	9 (29.0)	11 (18.6)	

‡Age at exposure is age at initial presentation not age at enrolment in the study.

†34 cases with missing data on total number of days hospitalised/ PSS/ ICU admission

Table 3 presents the results of the neurodevelopmental tests stratified by exposure group. Children in the paraffin group were compared to the control group as were the OPP to the control group separately. When compared to the control group, participants in the paraffin group performed significantly worse in fingertap repetition tests. On the CBCL, they had significantly higher internalising and total problem scores. They also scored significantly higher in 2 clinical syndrome (anxious/depressed, withdrawn/depressed) and 2 DSM (affective, anxiety) scales.

Similarly, when compared to the control group, participants in the OPP group performed significantly worse in finger tap repetition and grooved pegboard tests. They had significantly higher internalising, externalising, and total problem scores on the CBCL assessments. They scored significantly higher in 6 clinical syndrome scales (anxious/depressed, withdrawn/depressed, social problems, thought problems, aggressive and rule-breaking behaviour), 3 DSM scales (affective, anxiety and conduct) and 1 competence scale (school).

Table 3: Neurodevelopmental test results by exposure group

	Control N=29	Paraffin N=46	P value	OPP N=47	P value
Raven Total	13 (12-17)	14 (12-18)	0.451	15 (13-21)	0.132
Raven IQ	84 (71-97)	82 (74-90)	0.523	84 (71-90)	0.672
TROG	9.1 ± 3.6	10.1 ± 4.1	0.326	10.6 ± 4.6	0.133
Beery VMI	17.7 ± 3.4	17.9 ± 3.9	0.832	18.2 ± 4.4	0.610
Visual perception	15.2 ± 4.0	15.2 ± 4.2	0.974	15.8 ± 4.2	0.557
Motor coordination	15.7 ± 4.1	15.6 ± 4.2	0.897	15.0 ± 4.1	0.460
Digit span					
Total score	7.4 ± 3.2	7.9 ± 2.9	0.443	7.6 ± 2.7	0.752
Longest span forward	4 (4-5)	4 (4-5)	0.107	4 (4-5)	0.977
Longest span backward	2 (0-3)	2 (0-3)	0.407	2 (0-3)	0.434
Grooved pegboard					
PH time	56.5 (43.0-76.0)	64 (48-90)	0.153	83.5 (56-96.5)	**0.009**
NPH time	69.5 (51-91)	74.5 (60-98)	0.523	97.5 (64.5-129)	**0.030**
Fingertap repetition					
PH completion	20.5 (14-27)	13.5 (11-15)	**<0.001**	13 (11-16.5)	**0.001**
NPH completion	23 (13-28)	13 (11-17.5)	**0.004**	14 (12-22.5)	0.058
Fingertap sequences					
PH completion	19 (16-24)	17 (15-20)	0.118	16 (14-20)	0.070
NPH completion	16.5 (15-21)	16.5 (13-20.5)	0.621	18 (13-24)	0.789
CBCL					
Summary scales					
Internalising	3.5 (2-9)	9 (6-13)	**0.001**	11 (7-15)	**<0.001**
Externalising	6.5 (4-14)	12 (4-17)	0.402	15.5 (6-24)	**0.009**
Total problem score	26 (15-37)	38 (23-57)	**0.032**	51 (29-76)	**<0.001**
Clinical syndrome scales					
Anxious/depressed	1.5 (0-4)	4 (2-6)	**0.005**	5 (3-8)	**<0.001**
Emotionally reactive	-	4.7 ± 2.6	-	3 ± 1	-
Withdrawn/depressed	1 (0-2)	2.5 (1-4)	**0.030**	3 (1-5)	**0.003**
Somatic	1 (0-3)	3 (1-5)	**0.050**	2 (1-5)	0.078
Withdrawn	-	1.6 ± 1.2	-	2 ± 2.6	**-**
Social problems	3 (2-5)	4 (1.5-5)	0.970	5 (3-10)	**0.022**
Attention problems	2.5 (1-8)	4.5 (2-7)	0.318	5 (3-10)	0.112
Sleep problems	-	4 ± 1.1	-	2.3 ± 2.3	-
Thought problems	1 (0-4)	2 (1-4)	0.276	4 (2-8)	**0.002**
Aggressive behaviour	4 (2-11)	7.5 (3-13)	0.342	12 (5-20)	**0.009**
Rule-breaking behaviour	2 (1-4)	2 (0-5)	0.867	4 (2-6)	**0.017**
DSM scales					
Affective	1.5 (0-3)	3 (2-6)	**0.006**	4 (2-6)	**0.001**
Anxiety	0.5 (0-1)	2 (1-4)	**0.004**	2 (1-3)	**0.002**
PDD	-	2.7 ± 1.5	-	4.7 ± 4.5	**-**
Somatic	1 (0-2)	1 (0-3)	0.179	1 (0-3)	0.207
ADHD	3 (1-6)	5 (3-7)	0.183	5 (3-9)	0.066
Oppositional DD	2 (1-4)	2 (1-4)	0.982	4 (1-6)	0.142
Conduct	2 (1-4)	2.5 (1-6)	0.867	5 (2-10)	**0.015**
Competence scales					
Activities	6 ± 3.0	5.8 ± 3.0	0.860	6.4 ± 2.4	0.547
Social	7.2 ± 1.7	7.1 ± 1.8	0.864	6.7 ± 2.3	0.424
School	5 (4-6)	5 (3.5-6)	0.459	4 (3-5)	**0.031**

Data are presented as mean ± standard deviation or median (interquartile range) as appropriate. For normally distributed data, Student's t-test was used. For data that were not normally distributed, Wilcoxon rank-sum test was used. Tests were conducted at a 5% level of significance.

Table 4: Multivariate analysis†

	OPP vs Control (n=68)				Paraffin vs Control (n=75)				OPP vs Paraffin (n=85)			
	Unadjusted OR	**P value**	**Adjusted OR**	**P value**	**Unadjusted OR**	**P value**	**Adjusted OR**	**P value**	**Unadjusted OR**	**P value**	**Adjusted OR**	**P value**
Grooved pegboard												
PH time	3.6 (0.9-13.9)	0.066	3.6 (0.9-14.7)	0.078	2.0 (0.5-8.1)	0.345	1.9 (0.5-8.1)	0.375	1.8 (0.7-4.8)	0.228	2.2 (0.7-7.2)	0.185
NPH time	5.8 (1.5-22.3)	**0.010**	9.4 (2.0-43.6)	**0.004**	1.2 (0.3-5.3)	0.800	1.3 (0.3-6.5)	0.737	4.8 (1.7-13.7)	**0.003**	5.6 (1.5-21.0)	**0.011**
Fingertap repetition												
PH	6.2 (1.3-29.9)	**0.023**	7.0 (1.4-35.3)	**0.019**	6.2 (1.3-29.9)	**0.023**	6.1 (1.2-30.2)	**0.026**	1 (0.4-2.4)	1.000	1.2 (0.4-3.5)	0.771
NPH	1.6 (0.5-4.7)	0.436	1.2 (0.3-4.0)	0.781	1.7 (0.6-5.2)	0.334	1.5 (0.5-4.9)	0.478	0.9 (0.4-2.2)	0.821	1.1 (0.4-3.5)	0.820
Fingertap sequences												
PH	2.2 (0.7-6.9)	0.189	1.7 (0.5-6.1)	0.399	1.2 (0.4-4.1)	0.731	0.8 (0.2-3.0)	0.736	1.8 (0.7-4.5)	0.240	4.5 (1.1-18.0)	**0.031**
NPH	2.1 (0.7-6.3)	0.185	1.6 (0.4-5.4)	0.483	1.3 (0.4-3.9)	0.698	0.8 (0.2-3.0)	0.790	1.7 (0.7-4.1)	0.259	2.2 (0.6-7.7)	0.218
CBCL												
Summary scales												
Internalising	6.7 (0.8-55.6)	0.078	6.9 (0.8-59.0)	0.078	5.3 (0.6-44.8)	0.123	5.1 (0.6-43.6)	0.134	1.3 (0.5-3.2)	0.635	0.9 (0.3-2.8)	0.824
Externalising	7.4 (0.9-61.5)	0.063	6.3 (0.7-53.6)	0.095	3.6 (0.4-30.9)	0.246	3.0 (0.3-26.4)	0.326	2.1 (0.8-5.6)	0.147	1.6 (0.5-5.6)	0.444
Total problem score	10.9 (1.3-89.4)	**0.026**	11.1 (1.3-96.1)	**0.029**	3.6 (0.4-30.9)	0.246	2.9 (0.3-25.9)	0.340	3.1 (1.2-8.0)	**0.023**	4.4 (1.2-15.9)	**0.026**
Clinical syndrome scales												
Anxious/depressed	9.1 (1.1-74.5)	**0.040**	10.8 (1.3-92.1)	**0.029**	4.7 (0.6-39.9)	0.154	5.3 (0.6-45.9)	0.129	1.9 (0.8-4.9)	0.168	1.5 (0.5-4.6)	0.471
Withdrawn/depressed	3.5 (0.7-17.3)	0.130	3.5 (0.7-18.2)	0.136	1.3 (0.2-7.4)	0.775	0.9 (0.2-5.8)	0.944	2.7 (0.9-8.5)	0.091	4.6 (0.9-22.5)	0.062
Somatic	n/a				0.9 (0.3-2.4)	0.797	0.8 (0.3-2.2)	0.650	1.1 (0.4-3.1)	0.797	1.1 (0.3-3.6)	0.889
Social problems	4.3 (0.9-21.2)	0.074	4.5 (0.9-23.4)	0.076	0.7 (0.1-4.8)	0.741	0.7 (0.1-4.6)	0.666	5.9 (1.5-22.5)	**0.009**	12.6 (2.1-77.1)	**0.006**
Thought problems	n/a				0.5 (0.2-1.3)	0.132	0.4 (0.1-1.3)	0.136	2.2 (0.8-6.3)	0.132	2.4 (0.7-8.4)	0.183
Aggressive behaviour	7.4 (0.9-61.5)	0.063	6.4 (0.7-54.4)	0.091	4.1 (0.5-35.3)	0.194	3.5 (0.4-30.8)	0.251	1.8 (0.7-4.7)	0.232	1.1 (0.3-3.9)	0.824
Rule-breaking behaviour	9.1 (1.1-75.3)	**0.040**	7.0 (0.8-62.3)	0.080	4.1 (0.5-36.3)	0.204	2.1 (0.2-20.6)	0.531	2.2 (0.8-6.3)	0.132	3.1 (0.7-13.4)	0.139
DSM scales												
Affective	7.4 (0.9-61.5)	0.063	7.4 (0.9-63.4)	0.068	6.0 (0.7-50.1)	0.098	6.6 (0.8-56.2)	0.084	1.2 (0.5-3.1)	0.644	0.9 (0.3-2.6)	0.791
Anxiety	4.7 (0.6-39.9)	0.154	6.8 (0.8-60.0)	0.085	4.7 (0.6-39.9)	0.154	4.9 (0.6-43.3)	0.151	1 (0.4-2.7)	1.000	1.0 (0.3-3.3)	0.999
ADHD	3.5 (0.7-17.3)	0.125	3.6 (0.6-19.8)	0.145	1.0 (0.2-5.6)	0.978	0.6 (0.1-4.1)	0.631	3.6 (1.2-11.0)	**0.026**	3.5 (0.8-14.7)	0.093
Conduct	3.9 (0.8-19.2)	0.098	2.8 (0.5-15.2)	0.241	1.0 (0.2-6.1)	1.0	0.4 (0.1-3.3)	0.431	3.9 (1.1-13.1)	**0.030**	6.6 (1.2-36.1)	**0.029**
Competence scales												
Activities	2.4 (0.5-12.4)	0.287	2.5 (0.5-13.7)	0.280	1.9 (0.4-10.4)	0.444	1.2 (0.2-7.0)	0.875	1.3 (0.4-3.7)	0.682	1.8 (0.5-6.8)	0.399
Social	0.7 (0.2-2.8)	0.611	0.7 (0.2-2.8)	0.569	0.9 (0.2-3.6)	0.891	0.9 (0.2-3.8)	0.883	0.8 (0.2-2.5)	0.662	0.9 (0.2-3.7)	0.900
School	0.2 (0.03-1.3)	0.092	0.4 (0.05-2.8)	0.348	0.8 (0.2-3.3)	0.760	1.9 (0.3-10.3)	0.473	0.3 (0.05-1.5)	0.132	0.4 (0.04-3.6)	0.390

†Each row presents results of the logistic regression model for each neurodevelopment test outcome. Outcomes were dichotomised as highest quartile (Q4) compared to quartiles 1-3. Separate models are presented comparing the paraffin and OPP to the control group (control group used as reference category); as well as the paraffin to the OPP group (paraffin group used as reference category). For each comparison, we present 2 models: unadjusted and adjusted for sex, age, and socioeconomic status. For the comparison of the paraffin to OPP group the adjusted model was adjust for sex, age, socioeconomic status and poisoning severity.

Table 4 presents the results of the multivariate analysis. In the comparison of the OPP and control groups, the OPP group performed significantly worse in both the unadjusted and adjusted models for grooved pegboard (NPH), fingertap repetition (PH), total problem score and CBCL anxious/depressed clinical syndrome scale. As well as the unadjusted model for rule-breaking clinical syndrome scale.

In the comparison of the paraffin and control groups, participants in the paraffin group performed significantly worse in both the unadjusted and adjusted models in the fingertap repetition for the preferred hand (PH).

In the comparison of the OPP and paraffin groups, the OPP group performed significantly worse in both the unadjusted and adjusted models for grooved pegboard (NPH), total problem score, social problems clinical syndrome scale, ADHD and conduct DSM scales. We adjusted for severity of poisoning in the multivariate analyses comparing OP-poisoned and paraffin-poisoned groups and observed that the ORs remained significant and even increased in some cases.

Discussion

In this study we observed significant associations between acute poisoning with OP and long-term deficits in fine motor speed and control, childhood behaviour and social functioning, controlled for age, sex, socioeconomic status and severity. Higher ORs in the OP poisoned group (compared to controls) were observed in 19 out of 21 neurodevelopmental test outcomes. Four of these were significant: grooved pegboard (NPH), fingertap repetition (PH), CBCL total problem score and anxious/depressed clinical syndrome scale (Table 4). These deficits reflect impaired motor functioning, reduced motor speed, inattention, behavioural problems, and emotional difficulties.

Several studies have reported the occurrence of long term neurobehavioural impairments following acute OP poisoning. [17,49-55] Various mechanisms have been postulated for these long term impairments, however, it is not entirely clear if they are due to hypoxia in the acute phase of poisoning or due to subsequent neuroinflammation, oxidative stress or altered gene expression.[51,56-60] To determine whether brain hypoxia associated with poisoning may have been the primary reason for deficits, we included a group of children poisoned with paraffin (non-OP poisoned controls). The comparison of OP-poisoned children to paraffin poisoned children generated a similar pattern of higher ORs in the OP poisoned group for 18 out of 21 neurodevelopmental test outcomes, albeit with slightly lower ORs. Six of these differences were significant namely: grooved pegboard (NPH), fingertap sequences (PH), CBCL total problem score, social problems clinical syndrome scale, and ADHD and conduct DSM scales. Moreover, there were no significant associations in a negative direction for either set of comparisons.

Two other studies have shown similar impairments in attention and behavioural problems in OP exposed children .[27,61] Kofman et al, conducted a study where they also compared OP-poisoned children to non-poisoned controls and included a second comparator group of kerosene-poisoned children. They found deficits in inhibitory motor control (which are suggestive of attention problems), that were more marked

in OP-poisoned compared to kerosene-poisoned children.[27] Ruckart et al evaluated the long-term neurobehavioural effects of environmental methyl parathion (an OP insecticide) exposure in children. Compared to unexposed children, they reported subtle changes in attention as well as parental reports of impulsive behaviour, anger, problems interacting with other children and conduct disorder among OP exposed children.[61]

Apart from the aforementioned study by Kofman et al,[27] there are limited data in the literature on long term neurodevelopmental effects of acute OPP in children. Most studies on long term neurodevelopmental outcomes in children have typically evaluated effects of low dose exposure to OPs, as opposed to high dose exposure that occurs in acute poisoning, and have focused on pre-natal exposures. A systematic review by Sapbamrer et al concluded that there was considerable evidence that prenatal exposure to OPs contributes to childhood neurodevelopmental disorder but that there was limited and less conclusive evidence on effects resulting from postnatal exposure.[62]

Other studies have reported that prenatal exposure to OP was associated with attention problems and ADHD.[31,63,64] We also observed significantly higher ORs among children with OPP compared to paraffin poisoning in the ADHD and conduct DSM scales. Additionally, some significant effects, not evident in the comparison with normal controls emerged: ADHD and conduct DSM scales. This may suggest that these are effects are not simply the effect of hypoxia related to the acute incident but specific to OP poisoning.

We adjusted for severity of poisoning in the multivariate analyses comparing OP-poisoned and paraffin-poisoned groups and observed that the ORs remained significant and even increased in some cases. Whether the impairments we observed are due to hypoxia in the acute phase cannot be conclusively excluded. However, use of a non-OP poisoning control and adjusting for poisoning severity does suggest a specific effect of OP poisoning. For example, the long-term neurologic and neuropsychiatric impairments of OP poisoning could be a result of irreversible progressive neuronal cell death, neural loss and axonal degeneration triggered by excessive stimulation of cholinergic receptors in the acute phase of poisoning.[65]

Our findings were consistent with those from other studies that reported delays in gross and fine motor skills in children prenatally exposed to OP.[9,20,63] Several other studies have found associations between postnatal OP exposure and decreased fine motor control, problem solving psychomotor and social development.[9,18,66] In contrast to ours, other studies have reported that pre- and postnatal exposure was associated with poorer verbal comprehension, problem solving, visuomotor integration and IQ.[9,67,68]

These findings suggest that children surviving OP poisoning exhibit adverse impacts on various neurodevelopmental outcome measures. These effects are not merely the result of brain anoxia but appear to be specific to OP poisoning. It is possible that the differences between OP and paraffin could be due to the fact that OP-poisoned children were slightly older.

Limitations

This study has some limitations. Firstly, we had a lower than anticipated response rate for non-poisoned controls which meant we could not do a matched analysis as initially hoped. We may have underestimated our sample size estimates, and our small sample of non-poisoned controls size may have made it challenging to detect subtle deficits in fine motor control or behaviour. The unadjusted ORs for ADHD and conduct DSM scales were similar in the comparisons of OPP to both non-poisoned and paraffin-poisoned controls. However, they were not statistically significant in the former. This may have been due to the smaller sample of non-poisoned controls. Despite this, we were still able to show a consistent pattern of associations with both statistically significant differences for many outcomes and increased measures of effect for others in a pattern suggesting significant adverse neurodevelopment impacts.

Secondly, there are multiple factors that influence child neurodevelopment including social factors in the home environment. While we matched cases on age, sex, language, and date of admission, to minimise effect of any confounders, we recognise that there may have been other confounders which we were either unaware of or unable to measure. We matched participants on age at enrolment due to missing data, whether or not this had an effect is not clear. Children known to be HIV positive were excluded from the study. We acknowledge that in our context, this represents an important population sub-group, in whom these impacts may potentially be worse.

Conclusion

This study provides valuable insights on the long-term neurodevelopmental impacts of children surviving acute OP poisoning. It confirms that OP poisoning in children can leave them with varying degrees of neurodevelopmental deficit in the domains of motor functioning and speed, behavioural problems, attention and emotional wellbeing. These impacts appear to be specific to OP rather than the poisoning. It highlights the need for long term follow up, neurodevelopmental assessment and support of OP-poisoned children into adolescence. Further research using a standardised test such as the Griffiths mental development scale (Griffiths III) that assesses all aspects of development and includes children below three years could provide a more holistic view of the burden of neurodevelopmental deficits. The impacts of poor early child development and inappropriate OP use will be felt throughout society. These impacts span multiple sustainable development goals (SDGs) from health and wellbeing and quality education to responsible production, consumption and industry innovation.[69]

Furthermore, it emphasizes the need for more effective prevention measures. These should encompass phasing out of highly toxic pesticides, such as most organophosphates, to be replaced with less harmful agents, as well as prevention of the sale and use of illegal pesticides.

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Supplementary Material

Table 5: Correlation between socioeconomic variables

Variable	Employment	Maternal education	Housing
Maternal education	0.689 (p<0.001)		
Housing	0.689 (p<0.001)	0.901 (p<0.001)	
No of dependants	0.806 (p<0.001)	0.853 (p<0.001)	0.747 (p<0.001)

Appendix 1: Research protocol

Background

Pesticides are any "*substances, or mixture of substances of chemical or biological ingredients intended for repelling, destroying or controlling any pest, or regulating plant growth*".[1] They are widely used vector control and agriculture, but are highly toxic to humans.[1,2] Organophosphates are a class of commonly used pesticides.

Organophosphate poisoning (OPP) is a significant cause of both morbidity and mortality in developing countries. The World Health Organization (WHO) estimates that approximately 243 000 deaths were caused by unintentional poisonings in 2002 of which 65% occurred in developing countries.[3] In South Africa (SA), pesticide poisoning was the most common non-drug chemical exposure accounting for 18% of all poison centre consultations.[4] Of these cholinesterase inhibitors were the most commonly reported pesticide exposure.[4] Additionally, acute poisonings are a key reason for admission to hospital and particularly intensive care units.[5-7] A 2013 toxicovigilance survey of acute poisoning admissions to a tertiary hospital in the Western Cape found cholinesterase inhibitor poisonings to be the most frequent reason for admission to the intensive care unit (ICU).[7]

Children in particular bear a significant burden, accounting for 40% of all pesticide poisoning cases and reported to the poison centre.[4] Exposure of children to pesticides in rural and urban homes[8] as well as poisoning of children by pesticides in rural farming communities[9] remains a major public health challenge, not only globally[10] but particularly in South Africa.[11]

Pesticide poisoning is a Notifiable Medical Condition in SA[12] and a total of 12,364 cases of pesticide poisoning cases were reported between the years 2000 – 2008. Of these, 498 cases were from the Western Cape.[13] Although acute OPP cases reported annually have risen substantially from between 100 and 200 acute OPP cases per annum in the two decades,[14] there is extensive evidence of under-reporting of pesticides poisoning.[15]

In the acute phase, organophosphates inhibit esterase enzymes especially acetylcholinesterase resulting in accumulation of acetylcholine, overstimulation of these receptors at neuromuscular junctions, synapses and end organs and subsequent autonomic, neuromuscular and central nervous system clinical features.[16,17] However, in addition to the acute manifestations of OPP, there have been long-term neurological and neuropsychological deficits documented in survivors of acute OPP.[17-22] Adults with a history of OPP have been shown to develop delayed polyneuropathies, characterised by deficits ranging from sensory abnormalities and weakness to even paralysis.[19] Other studies have also found that this population has had increased symptom prevalence, deficits in cognitive, personality and psychomotor function, decreased vibration sensitivity, and motor dysfunction.[20-26] Changes in affect have also been reported in adult survivors.[21]

In children, there have been a number of studies examining the consequences of pre-natal exposure in utero, or low-levels early childhood exposures to organophosphate and organochlorine pesticides, which have suggested deficits in short-term memory, increased reaction time, impaired mental development, impaired neonatal reflexes and pervasive emotional or developmental difficulties.[27-29] However, the literature describing the long term effects of acute poisonings in children is not as extensive, and certainly not as extensive as that existing for adults. There are case reports which have shown late-onset distal polyneuropathies in 2 children,[30] impairments in balance, reaction time, colour vision, trails making and grooved pegboard test in 3 children,[31] as well as 9 children with subsequent difficulties with both motor inhibition and verbal learning memory tasks.[32] The latter study focused on functions related to the forebrain nuclei. Neuro-anatomically, the basal ganglia comprise a cholinergic rich brain area that has also been demonstrated to be sensitive to in the acute phase to organophosphate neurotoxicity.[33] Although the basal ganglia have extensive neural connections to frontal lobes mediating higher cognitive and executive functions, which may explain the findings of Kofman et al,[32] there have been no studies in children examining the long term effect of acute OPP on other functions of the basal ganglia such as movement, balance and co-ordination.

A unique opportunity exists in SA given that there is a large available sample size of paediatric survivors of acute OPP who can be tested for similar and other neurological deficits. Of acute pesticide poisoning cases notified countrywide, approximately 40% are children 12 years or younger.[4] Previous studies have found under-reporting to be 80% or more,[15] which would then suggest that there might be as many as 300 to 500 cases of pediatric OPP per year. A recent pilot study has already identified 80 cases of children with acute pesticide poisoning who were admitted to Red Cross Children's Hospital in Cape Town, South Africa over the course of 18 months, which would be approximately four to five children per month.[34]

The health of children is recognised as one of South Africa's social priorities through the enactment of a range of policies and programmes aimed at protecting children from exploitation and promoting their development. For example, the first public policy announced by former President Mandela in 1994 was that of free health care for children. To a large extent, this commitment to children reflects South Africa's constitutional imperative that in all matters affecting the child, the child's best interests should come first. Improving child health and reducing exposure to hazardous chemicals also features strongly in the Sustainable Development Goals.[35] The question of protecting child health and their development, therefore, remains both a national and international priority. This concern for children is largely borne out of recent estimates on child deaths. A recent child death review pilot of found that non-natural deaths accounted for over a third (35.4%) of all deaths at Salt River mortuary (a mortuary that serves the Cape Metro) in 2014.[36]

This concern for children is, to a large extent, borne out in Burden of Disease assessments, which highlighted childhood illness as one of the five major contributors to the Burden of Disease in the WC Province.[37]

This study therefore offers an opportunity to identify a novel and, as yet poorly characterised contributor to the Burden of Disease in SA, as well as adding to the international body of knowledge in this field. These findings will also improve our understanding of the risk associated with environmental hazards.

Aim

To identify whether children in SA surviving acute OPP suffer any long-term neurodevelopmental impairment. This will enable better delineation of the full impact of the acute pesticide poisoning, as well as the long term needs of survivors. Data for the current study has already been collected and this protocol serves to present the methods for data analysis and reporting, as presented in the objectives listed below.*

Objectives

- To determine the performance of South African children surviving acute episodes of OPP on a standard set of paediatric neurodevelopmental batteries.
- To compare the performance of OPP survivors to two control groups of children drawn from Red Cross Children's Hospital (RXH), matched for age, gender, and home language.

Research hypothesis

Children who survive an acute OPP have subsequent neurological or neurodevelopmental deficits that present after the acute episode.

Methods

Study design

This was a case control study in which there was an index population of OPP survivors and two control groups in which neurological and neurodevelopmental performance will be compared.

Study population

Children admitted to the RXH with a diagnosis of acute OPP from 1 January 2001 to 31 December 2011 formed the index group. Children aged 3 to 12 at the time of enrolment were selected. This age group was selected based on the applicability of the neurodevelopmental tests to be utilised. This group was identified by a review of hospital records, and search of the Poison Centre database looking for cases coded as: pesticide poisoning; or poisoning (with subsequent review of the chart to specify type of poisoning).

There were two **control groups** of children drawn from a population of children admitted to RXH:

- Children admitted to hospital for poisoning where the agent was not a pesticide.

* Dr Mureithi acquired the data after collection by the study team. The methods presented below generated the data set on which the analysis and write up of the dissertation is based. She is primarily responsible for data analysis.

- Children admitted for conditions other than poisoning.

Inclusion and exclusion criteria

Inclusion and exclusion criteria for the index and control groups were as follows:

Inclusion Criteria for ALL children:

- Between ages of 3 to 12 years old at time of enrollment
- Admission to RXH between 1 January 2001 to 31 December 2011.
- First language English, Afrikaans, or Xhosa

Index Group:

Inclusion criteria:

- A positive history of acute poisoning with organophosphates, as described in available documentation, based on the following criteria:
    - Diagnosis of acute OPP documented by any physician involved with patient's care, based on clinical signs and symptoms.
    - Documented response and/or improvement to atropine or pralidoxime constituted, but was not necessary, for a positive diagnosis.
    - Documented single red blood cell (RBC) or plasma cholinesterase level during time of admission that was positive constituted, but was not necessary, for a positive diagnosis.
        - A negative RBC cholinesterase level does not exclude the diagnosis in the presence of documented pernicious anemia, hemoglobinopathies, use of antimalarial drugs, or oxalate blood tubes.
        - A negative plasma cholinesterase level does not exclude the diagnosis in the presence of documented liver dysfunction, low-protein conditions, neoplasia, hypersensitivity reactions, use of succinylcholine, codeine, and morphine, and known genetic deficiencies of cholinesterase.
        - A negative cholinesterase level in the context of a documented clinical picture consistent with organophosphate poisoning will be considered a positive diagnosis.

Exclusion criteria:

- Known health or other conditions resulting in a neurodevelopmental problem or disability (i.e., developmental disability due to any other cause, e.g., cognitive, motor, sensory or behavioural disability, chronic illness).

- Documented or reported interim history of other acute neurologically impairing event (including, but not limited to meningitis, head trauma with intracranial bleed or diffuse axonal injury);
- History of epilepsy;
- Known mental health conditions or psychiatric illness such as depression, anxiety disorder;
- Any child known to be HIV positive on records (no active HIV testing to be undertaken);
- Any underlying medical, neurologic, or behavioural condition that might influence performance in neurodevelopmental testing (including, but not limited to, cerebral palsy, autistic spectrum disorder).

First control group (poisoned but not by pesticide)

Inclusion criteria:

- A child admitted to RXH for a poisoning involving a known agent other than pesticides (e.g., paraffin, paracetamol, etc.) within the time period specified.

Second control group (not poisoned at all):

Inclusion criteria:

- A child admitted to RXH for a condition other than poisoning (not including any condition warranting exclusion – see below) within the time period specified.

All controls:

Exclusion criteria:

Exclusion criteria for the controls were the same as for index cases. The following additional exclusion criteria were applied:

- Any previous history of acute or chronic organophosphate poisoning, defined as one or more of the following were grounds for exclusion: poisoning on patient history, poisoning and seen by a physician, or poisoning and admitted to hospital.
- Known health or other conditions resulting in a neurodevelopmental problem or disability (i.e., developmental disability due to any other cause, e.g., cognitive, motor, sensory or behavioural disability, chronic illness).
- Documented or reported interim history of other acute neurologically impairing event (including, but not limited to meningitis, head trauma with intracranial bleed or diffuse axonal injury).
- History of epilepsy;
- Known mental health conditions/ psychiatric illness such as depression, anxiety disorder;
- Any child known to be HIV positive on records (no active HIV testing was undertaken);
- Any underlying medical, neurologic, or behavioural condition that might influence performance in neurodevelopmental testing (including, but not limited to, cerebral palsy, autistic spectrum disorder).

The **control** children were matched to cases on a 1:1 basis for the following variables:

- Date of admission: Within 6 months of admission.
- Age: Within 6 months
- Gender
- First language

Sampling

Sample size calculation was based on the Test for the Reception of Grammar (TROG), which have an IQ equivalent mean of 100 with a SD of 15, and numerical scales which have a mean of 10 with a SD of 3. An estimated 50 patients from the index population and 50 matched controls were estimated to provide an alpha of 0.05, power of 0.92 with a two-sided test, and allowing for a detection of an estimated difference of means of 10 points on the cognitive scales. The same sample size would allow for the same alpha and power to detect a 1-point difference in the numerical scales between two groups. Over the course of 9 years, over 500 cases of pesticide poisoning at RXH were expected, of which approximately 50-60% would be cases of organophosphate poisoning (Bloch et al, 2010). Considering attrition and exclusion criteria to reduce that to 25%, 50 to 60 index cases meeting inclusion criteria were expected.

Study procedures

The study team identified from hospital records a set of 8 seemingly eligible controls for each case based on matching criteria above – 4 controls for the poisoned (not by pesticides) group and 4 for the non-poisoned controls. These formed a pool of controls against which to match cases in the complete sample. Controls selected but not used for a particular case remained eligible for selection as a control for other cases if they met the matching criteria. A research assistant fluent in the patient's language assisted in contacting family members using details recorded in the patient folders to arrange the follow up assessment. The Poisons Centre at RXH was approached to identify children in both the index group and in the non-organophosphate poisoned control group.

Neurodevelopmental Assessment

Once the potential index and control group candidates were identified, the investigator or a delegate approached the patients and controls and their families or primary caregivers. They were invited by a member of the research team to attend a clinic for neurodevelopmental assessment and asked to participate if they agreed to informed consent, and assent for children 7 years or older. If consent and/or assent was obtained, each patient was examined by a trained test administrator and tested at one point in time during the recruitment period between 1 October 2012 to 31 June 2013. Based on experience in the pilot study, the anticipated non-response (mainly untraceable and less commonly refusal or having exclusion criteria) was expected to be about as 75%.

Patients were tested and interviewed in their primary language, and by a trained tester who was blinded to the exposure (i.e., index vs. either control group) and fluent in the patient's primary language. Tests were translated into Afrikaans or Xhosa, back translated and pilot tested.

Each patient was evaluated using five standardised tests. The time required to complete the entire battery of tests was between one to two hours. A report summarising the test battery results was prepared for each child's caregiver.

1. ***Raven Colored Progressive Matrices[38]:*** This cross-cultural test assesses non-verbal problem solving based on matching components of patterns and is normed for children aged 3 years 6 months to 11 years. It has been used widely in cognitive research in developing countries. This test relies on percentile scores for interpretation. Above the 95th centile is intellectually superior; above the 75th centile is above average intellectual capacity; between the 25th and 75th centiles is intellectually average; below the 25th centile is below average intellectual capacity and at or below the 5th centile is intellectual impairment. It is suited for children 3 to 14 years old.
2. ***Test for the Reception of Grammar (TROG)[39]:*** This test consists of identification of 80 items depicting increasing complexity of syntactic and lexical grammar, and has been shown to be useful as a measure of verbal abilities in children with developmental disabilities. Because TROG is designed to provide qualitative information about which aspects of grammar give difficulty, as well as an overall score, TROG items are grouped in blocks of four. The standard score is 100 and the S.D. 15. Scores may also be expressed as a percentile. The TROG-2 is a revised test version with new expanded age norms from 2 years to young adulthood.

The Raven and the TROG are tests of non-verbal and verbal capacity, respectively, for children. They have been successfully used to evaluate cognitive function in the South African population in previous studies (e.g., children with fetal alcohol syndrome) as proxies for more extensive IQ tests, such as the Junior South African Individual Scales (JSAIS) if aged 3 to less than 8 years old or the Senior South African Individual Scales – Revised (SSAIS-R) if 7 years old to less than 17 years. The RAVENS and TROG tests are appropriate for the nature of our study and have the advantage of being less time and labour intensive and can be conducted effectively by a trained individual without a psychometry or psychology background.

3. ***Beery-Buktenica Visual Motor Integration, Visual Perception and Motor Co-ordination[40]*** : The Beery Developmental Test of Visual Motor Integration (VMI) assesses visual-motor integration by requiring the child to copy 24 geometric figures. This test is constructed to measure the level of visual-motor development from the age of three to 14 years. Each design is harder than the last and testing is continued until three consecutive figures are failed. There are two supplemental tests: the VMI Visual Perception and the VMI Motor Coordination tests. These tests use the same stimulus forms and thus are comparable to the main test. Normative data are available for all ages. The Beery Buktenica has been extensively used in research studies and provides a sensitive

measure of fine motor problems in children. The overall VMI score is a good predictor of reading ability.

4. ***Digit Span:*** The Digit span, or memory for digits test is a test of attention and working memory, which are components of the wider domain of executive function. Executive functioning is influenced by the basal ganglia, thus may be impaired in children with OPP. The test has a forward and backward phase. The tester first reads a sequence of numbers forwards which the child repeats in the same manner. If no errors are made the span is increased until two sets of responses at the same span are incorrect. The child is then instructed to repeat a sequence numbers, but backwards and the span is again increased until 2 consecutive errors are made at the same span. This test shows good reliability and validity and is commonly included in many IQ batteries. For children 3 years to less than 8 years, the Memory for Digits test from the Junior South African Individual Scales (J-SAIS) will be used. For children aged 8 years to below 17 years, the same test from the Senior South African Individual Scales-Revised will be used.

5. ***Grooved Pegboard Test*** *(Matthews & Klove, 1964)*: The Grooved Pegboard Test consists of a pegboard with 25 grooved slots. The child is required to place a grooved peg into each of the slots first with the right hand and then the left hand. The time to complete the task with each hand is recorded. Normative data are available for children ages five to 16 years. The test assesses fine motor dexterity and co-ordination. Administration time is approximately 8 minutes. This test assesses dexterity and handedness, useful in the context of OPP to elicit fine motor and hand speed deficit suggestive of extra-pyramidal tract problems, known to occur in chronic OPP.

6. ***Achenbach System of Emperically Based Assessment (ASEBA) Child Behavior Checklist questionnaire (CBCL).***[41] These scales make up a comprehensive evaluation of childhood behaviour and social functioning. Behaviour scales such as this are useful to capture behavioural characteristics, in particular those related to attention and concentration. The categorisations for problem behaviours are based on a normative sample of North American children and the scales have been extensively used in both research and clinical practice. The questionnaires will be administered to parents or caregivers. The CBCL has been translated into Afrikaans and Xhosa for use in SA.

   The CBCL has two versions: one for children 1.5 to 5 years, and another for 6 to 18 years of age. Children 3 to 5 years of age will receive the first version, and children 6 years to 18 years old will receive the second version. The form for younger children has 100 questionnaire items and the checklist for older children has 113 items. The CBCL measures a child's competence and problem areas as perceived by the parent or caregiver. There are three responses (not true, somewhat, or sometimes true, very true or often true) and eight syndrome scales (Withdrawn, Somatic Complaints, Anxious/Depressed, Social Problems, Thought Problems, Attention Problems, Delinquent Behavior, and Aggressive Behavior). In addition, for children aged 6 and older, three competence scales measure the child's perceived competence in activities, social, and school

settings. The eight syndrome scales are summarised in three scales: Internalizing (Withdrawn, Somatic Complaints, and Anxious/Depressed), Externalizing (Aggressive Behavior, Delinquent Behavior), and Total problem scales. The dependent measures are the summary scales (Internalizing, Externalizing, Total) and the eight problem scales. A more recent addition to the scales analysis allows for behaviour description in terms of DSM-oriented problems, namely Anxiety, Withdrawal, Somatic, Attentional, Hyperactivity, Impulsivity, Conduct and Oppositional problems. This scale would be useful in detecting any reported attentional deficits in children with OPP relative to controls.

Limitation of the test battery:

First, few internationally used neurodevelopmental and cognitive tests for children and adults have been normed in South Africa and other developing countries. Socio-economic deprivation has a negative impact on performance on most tests of cognition normed in developed countries, and the average test performance scores of typically developing children from lower income countries are below the average norms for children in higher income countries in which the test batteries are normed. For this reason, it is important to include a control group in cognitive studies of children undertaken in developing country settings.

Second, the battery was translated into, and administered in more than one language. There may inevitably be some loss of validity through translation of test items into other languages than that in which the test was originally developed. Our test results will also be compared across languages. To minimize these limitations, control and case children were matched for first language. Although only the Digit Span test has been normed for South African children, all five tests are currently being used on a regular basis in the clinical setting and other research studies in SA (Dr. Adnams 2007, personal communication).

Site preparation and participation of the health services

The Developmental Service at RXH approved the project. Under the leadership of Dr Kirsty Donald, this clinic provided a room for use by the study researcher and assistant to conduct the assessments. All the key services to whom referrals were made were contacted prior to study commencement. The School of Public Health and Family Medicine at UCT provided office space and a telephone/ fax for contacting family members and organising follow up appointments. Approval was received for the study from the hospital management. The hospital information system and the Poisons Information Centre was approached for listings of patients that meet the inclusion criteria as outlined earlier, including children who were admitted with poisoning by other than pesticides.

Data Management

Results were recorded on tester examination forms; and scored and checked data were transferred to a PC synchronously with the testing. The part-time collection allowed the testers to score and enter their data in the same week or within three weeks of testing to enable timeous checking for data quality and corrections needed.

Data were stored electronically with electronic patient identifiers (in the event of referral needed or further details needed from the caregiver) but kept confidential. The listing of assigned numbers against study participants' names were kept separately and maintained securely and confidentially. The study researcher was responsible for oversight of the data quality and ensuring data were up to date.

Data analysis[†]

Statistical analyses will be performed using STATA version 13.0 (Statacorp, College Station, Texas). Demographics (age at time of testing, gender, race, socioeconomic status), and outcome measures (Raven, TROG, Beery-Buktenica Test, Digit Span, Grooved Pegboard Test, and Achenbach CBCL Scales) will be compared between the control group and the children with a history of acute OPP. Nominal data will be analyzed with χ^2 statistics. For data that are not normally distributed, the nonparametric Wilcoxon's signed rank test will be used. For normally distributed data, Student's t-test will be used. Tests will be conducted at a 5% level of significance. Covariates, which will be evaluated in subsequent logistic regression, will include the following: socioeconomic status, maternal education, race, gender, age at which acute OPP occurred, household member involved with work with organophosphates, proximity of household from potential organophosphate area of use, time elapsed since poisoning, severity of poisoning (i.e. type and duration of treatment, need for and duration of ventilatory support), and agent involved in poisoning. Children who experienced multiple episodes of OPP will be noted, and if there are sufficient numbers, a stratified analysis with this group will also be performed. These possible covariates will be explored, but we acknowledge the possibility that there may be insufficient data to do this in detail.

Patients who are not amenable to follow up for neurodevelopmental assessment (including, but not limited to, unable to contact for follow up, lack transportation to attend clinic) are not excluded, but will be considered non-responders. It is recognised that the absence of this population may limit generalisability, but we expect that there should be an even distribution of individuals in both the case and control groups who will be excluded for this same reason.

Variables such as race, socio-economic status, education, and illness severity (high care versus ICU admission) will be taken into account in analysis but will not be used for matching.

Ethical considerations

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki.[42] During the study, ethical research principles were adhered to and will continue to be upheld. The project was first approved on 21 December 2007 by the University of Cape Town Human Research Ethics Committee (HREC Ref: 468/2007). A renewal of the application will be made in light of the long delay in implementing the study.

[†] Future tense is used as protocol approval preceded analysis. Dr Mureithi is primarily responsible for analysis of data that were collected a priori.

Confidentiality

Patient information (e.g., test scores, demographics) were recorded on standardised sheets and stored in a locked cabinet in the School of Public Health and Family Medicine. Test scores were identified with an assigned number, rather than patient name or demographics. The only people with access to this data are the investigators and research assistants. This information will not be released with identifying details to anyone else outside of the study without the participant's written consent. Confidentiality will be maintained, and no participant will be identified in any of the project reports.

Risks

There was minimal risk associated with this study. There were no invasive tests nor samples collected from each child. The child may have experienced some distress and frustration if they found the tests or questions difficult, but otherwise, there would have been little else in the process to cause harm or discomfort. Informed consent was sought from all participants and all tests were conducted in their first language. Written consent was obtained from a parent or legal guardian and written, or verbal assent was obtained from children seven (7) years and older.

Benefits/ follow up to neurodevelopmental testing

Reports summarising results of each participant's performance were provided to parents. If neurodevelopmental problems or other ill-effects from poisoning were identified that will, or have impaired children's learning progress, parents were advised of appropriate local support mechanisms and resources.

Children with significant learning problems were referred to the Western Cape Department of Education School Psychological Services for appropriate intervention and support, consistent with Western Cape Department of Education policy for learners with special educational needs. Children who required educational support in the mainstream education system were brought to the attention of their own schools' educational support structures. Professor Adnams and Dr Donald have ample experience of liaison with the Education Department and used these networks to facilitate referral. Preschoolers with developmental problems were referred to the Developmental Clinic at RXH or appropriate local service.

If any participant scored a 1 or a 2 on questions #8 and/or #91 the CBCL, or spontaneously disclosed suicidal ideation, the interviewer (a non-clinician, was not expected to exercise clinical judgment) notified the collaborating paediatricians immediately for review and relevant mental health or general paediatric service referral as necessary. There are established routes of referral for general paediatric and mental health services in the Western Cape. The principal investigators received same-day notification of any child who required paediatric or mental health evaluation.

After assessment, a copy of the report was given to the child's primary caregiver (e.g., parent, grandparent, guardian) and the results explained to them. The study identified each child as scoring, "Average", "Above average", or "Below average" in each tested category. The interviewer would specify that relative to the rest of the population, their child scored the same, or above, or below what

most other children scored. The study team offered the caregiver the opportunity to send the report to the child's school where the study results may have been used as an adjuvant to the learner's school records and may hopefully have informed the school's general educational management of the child.

Compensation

Participating families were not paid for their participation. However, travel costs were reimbursed. Compensation for effort and time was given to participants in the form of a shopping voucher for a local supermarket to the effect of R 50 per visit.

Dissemination

The results of the study will be disseminated in the following ways:

- A report to the Western Cape Department of Health (WCDoH) will be provided 6 months after completion of the analysis.
- A presentation based on these results will be made to the weekly grand rounds at Red Cross Hospital to alert clinicians to the importance of long-term follow up of children who survive acute OP poisoning. An offer will be made to similar CME-type meetings at other institutions in the province (e.g., Tygerberg, Paarl, Somerset-West and Worcester Hospitals' paediatric services).
- The report will be shared with the coordinating clinician for paediatric services in the WCDoH.
- One of more articles will be written up for publication in a scientific journal.
- One or more presentations will be made on the findings, including a presentation at the Public Health Association of South Africa conference.
- The report will also be sent to other government stakeholders – local authorities such as the City of Cape Town, and the Boland, Eden, Overberg, and West Coast Municipalities; the Department of Agriculture; the Department of Labour – as well as NGOs concerned with child safety issues.
- Where appropriate, material from the research findings will be highlighted in educational media directed at child safety. The SOPHFM also runs a listserver and distributes a newsletter on pesticides for Southern Africa which will enable wider distribution of this information.

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Appendix 2: Ethics approval letter (original)

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: Lamees.Emjedi@uct.ac.za

21 December 2007

REC REF: 468/2007

Professor Leslie London
School of Public Health and Family Medicine
Falmouth Building
Medical School

Dear Professor London

LONG TERM EFFECTS OF ACUTE PESTICIDE POISONING ON SOUTH AFRICAN CHILDREN

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study

The study is considered minimal risk and was expedited in terms of Category 7 of the guidance relating to 45 CFR 46.110. The study is approved for one year and current approval expires on 21 December 2008.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

A/PROF. M. BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

pp

Faculty

Appendix 3: Ethics annual renewal

HUMAN RESEARCH ETHICS COMMITTEE
27 FEB 2023
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

UNIVERSITY OF CAPE TOWN
FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.01.2024
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	27/2/23

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	21 Feb 2023		
HREC REF Number	468/ 2007	Current Ethics Approval was granted until	31 Jan 2023
Protocol title	Long-term effects of acute pesticide poisoning on South African children		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?		☐ Yes	☑ No
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Professor Leslie London		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Appendix 4: Information sheet and consent form

Title of the research project:

Long-term neurodevelopmental outcomes of acute organophosphate poisoning in South African children.

Reference number: HREC 468/2007

Principal Investigator: Prof Leslie London

Address: Red Cross Children's Hospital and the Department of Public Health University of Cape Town

Contact number: (021) 406 6524

Your child is being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how your child could be involved. Also, your child's participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you or your child negatively in any way whatsoever. You are also free to withdraw him/her from the study at any point, even if you do initially agree to let him/her take part.

This study has been approved by the **Committee for Human Research at University of Cape Town** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

Organophosphate poisoning (OPP) is a significant cause of morbidity and mortality in developing countries. Most research on the neurological and neurobehavioural sequelae of OPP has been in adults and little is known about the neurodevelopmental outcomes of children who survive acute organophosphate pesticide poisoning (APP). Given the extent of APP in developing countries and in South Africa, the burden on children's neurodevelopment may be unrecognised. The long-term effects of APP that are described in children suggest cognitive, executive function and motor impairments that involve the brain basal ganglia. However, the precise neurobehavioural phenotype for long-term effects of OPP remains undetermined.

Why has your child been invited to participate?

Your child has been exposed to poisons and this study focuses on determining the long-term neurodevelopmental outcomes of acute organophosphate poisoning.

What will your responsibilities be?

Your child will be given an assessment of learning at the Red Cross Children's Hospital. The session will take 1-2 hours in total. There will only be one set of assessments.

Will your child benefit from taking part in this research?

Because your child has previously been exposed to poison we will be testing their developmental growth and dexterity to compare of they are on par with their peers. If there are any reasons for concern you and your child will be referred for further assessments.

Are there any risks involved in your child taking part in this research?

No. Your child may become bored and not find this enjoyable but they will experience no pain. If at any time they become upset and do not wish to continue, the task will be stopped.

Who will have access to your child's medical records?

Only members of the research team will have access to the data gathered here. All information will remain confidential and if the results of this study are published no participant will be identified. We may require access to your child's medical records. We will only ask for access to these records with your written permission.

Will you or your child be paid to take part in this study and are there any costs involved?

You or your child will not be paid to take part in the study, but your/your child's transport will be covered for the visit. There will be no costs involved for you if your child does take part.

Is there anything else that you should know or do?

- You can contact Prof London tel (021) 406 6524 if you have any further queries or encounter any problems.
- You can contact the Committee for Human Research at if you have any concerns or complaints that have not been adequately addressed by your child's study doctor.

Assent of minor

I (*Name of Child/Minor*)……. have been invited to take part in the above research project.

- The study doctor/nurse and my parents have explained the details of the study to me and I understand what they have said to me.
- They have also explained that this study will involve. I also know that I am free to withdraw from the study at any time if I am unhappy.
- By writing my name below, I voluntary agree to take part in this research project. I confirm that I have not been forced either by my parents or doctor to take part.

_____ _____

Name of child **Independent witness**

(To be written by the child if possible)

Declaration by parent/legal guardian

By signing below, I *(name of parent/legal guardian)* ……..……. agree to allow my child (name of child) ……..…. who is ……. years old, to take part in a research study entitled *(insert title of study)*

I declare that:

- I have read or had read to me this information and consent form and that it is written in a language with which I am fluent and comfortable.
- If my child is older than 7 years, he/she must agree to take part in the study and his/her ASSENT must be recorded on this form.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to let my child take part.
- I may choose to withdraw my child from the study at any time and my child will not be penalised or prejudiced in any way.
- My child may be asked to leave the study before it has finished if the study doctor or researcher feels it is in my child's best interests, or if my child does not follow the study plan as agreed to.

Signed at (*place*) ……..…….. on (*date*) ……..…….. 2013.

_____ _____

Signature of parent/legal guardian **Signature of witness**

Declaration by investigator

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understand all aspects of the research, as discussed above
- I did/did not use a translator (i*f a translator is used, then the translator must sign the declaration below)*.

Signed at (*place*) ……..…….. on (*date*) ……..…….. 2013.

_____ _____

Signature of investigator **Signature of witness**

Declaration by translator

I *(name)* ……..…… declare that:

- I assisted the investigator (*name*) ……..……. to explain the information in this document to (*name of parent/legal guardian*) ……...……... using the language medium of Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the parent/legal guardian fully understands the content of this informed consent document and has had all his/her questions satisfactorily answered.

Signed at (*place*) .....…..…….. on (*date*) ……...…….. 2013.

_____ _____

Signature of translator **Signature of witness**

I declare that:

I grant/do not grant the researcher permission to make my child's results known to my treating doctor

Signed at (*place*)……..on (*date*) …….. 2013

_____ _____

Signature of Participant Signature of Witness.

I declare that:

I grant/do not grant the researcher permission to access my child's medical records.

Signed at (*place*)……..on (*date*) …….. 2013

_____ _____

Signature of Participant Signature of Witness.

Appendix 5: Author guidelines for submission of manuscript to the Environmental Health Perspectives (EHP) journal

(https://ehp.niehs.nih.gov/authors/preparing-your-manuscript)

Aims and scope:

We publish high-quality original research, reviews and commentaries on all established and emerging disciplines that examine the relationship between the environment and human health.

General Guidance

Manuscripts should be as concise as possible without sacrificing clarity or limiting reproducibility. When appropriate, use active voice to avoid ambiguity. EHP covers all disciplines engaged in the broad field of environmental health science. Therefore, we ask authors to avoid jargon and define any terms that may not be universally recognized or consistently used.

Line Numbering

Enable continuous line numbering on all manuscripts (i.e., line numbers should NOT restart at 1 on each page). Manuscripts received without continuous line numbers will be returned to the author for revision before peer review.

Title Page

Include the following items in the order shown, beginning on the first page of the manuscript:

- Manuscript title
- Names of the authors, with the first name provided first
- Affiliations of all authors (department, institution, city, state/province, and country)
- Complete contact information for the corresponding author (name, email address, and postal address)
- Declaration of conflicts of interest

Symbols and Equations

Use MathType or Word's Equation Builder tool to generate mathematical expressions and equations, as well as any equation variables used within the text itself.

- Place simple expressions and equations in line. Present in-line equations on one line, and do not stack fractions.
- Place complex expressions and equations, including those with stacked fractions, on a separate line, and include a number in brackets (on the same line, to the right).
- Define all nonstandard elements, including superscripts and subscripts) if needed.
- Use bold text to represent vectors.

Footnotes

Do not use footnotes in the main manuscript text.

Research Articles

Research articles report original research results that are relevant to the relationship between the environment and human health, and that make a substantial advance in the field.

EHP strongly recommends that authors consult the following reporting guidelines while drafting manuscripts:

- For observational research studies, authors should consult an appropriate version of the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.
- For research articles involving animal subjects, authors must adhere to the ARRIVE (Animals in Research: Reporting *in Vivo* Experiments) guidelines for reporting animal research (Kilkenny et al. 2010; Tilson and Schroeder 2013).

Regarding papers that consider race or ethnicity:

- As noted by the *AMA Manual of Style* committee, "Continual review of the language used to describe race and ethnicity is critically important" (Flanagin et al. 2021). Thus, *EHP* may revise these guidelines as necessary. We recognize that these guidelines represent a change from prior journal practices, and we anticipate and welcome the conversation this may precipitate.
- Racial and ethnic groups should be described using terms used by study participants to describe themselves (e.g., if the study questionnaire options included Black do not describe participants as African American) and designated by capitalization (e.g., Black versus black). For additional guidance on reporting race and ethnicity, we recommend the APA Racial and Ethnic Identity Style Guidelines.
- Because race is a social construct, *EHP* generally will not consider research that proposes or assumes a genetic basis as the only explanation for racial health disparities (see Kaufman and Hajat 2021). If a genetic explanation is proposed as a basis for health disparities, this explanation should be framed in terms of genetic ancestry rather than "race" (Oni-Orisan et al. 2021).
- We urge investigators to develop and use methods to measure racism and/or racist policies and their effects directly whenever possible, instead of using race/ethnicity as a convenient proxy. We expect authors to be thoughtful in considering racism as an underlying reason for downstream impacts, rather than simply attributing health effects to race. Importantly, the absence of data does not imply that disparities in exposures and health do not exist.

Suggested Length

Suggested length is < 7,000 words, excluding the text in the abstract, references, tables, figure captions, acknowledgments, and Supplemental Material.

Title

The title should consist of ≤ 300 characters and should state the subject of the paper and include relevant information to help potential readers determine whether the paper might be related to their interests or needs. Relevant information includes the exposure(s) and outcome(s) assessed, and whether the study was observational or experimental. For epidemiological studies, consider key characteristics of the study population (e.g., gender, age, location, cohort) and design. For experimental studies, indicate the experimental model, including species or *in vitro* system(s). The title should not be a declarative statement of the study results or conclusions.

Abstract

Include a structured abstract of ≤ 300 words using the following headings: Background, Objectives, Methods, Results, Discussion. The abstract should not include references or any information that does not appear in the text of the manuscript. We recommend that authors indicate study names or sources of data that are integral to the study. Summarize major findings in a balanced manner, rather than focusing only on findings that support the study hypothesis.

Main Text Structure

Sections should appear in the following order:

- Introduction
- Methods
- Results
- Discussion
- References
- Tables
- Figure captions

Concise subheadings (≤ 8 words each) may be used to designate major topics within each of these sections. Subheadings should be used to organize information, but should not summarize or interpret results or conclusions.

Introduction

Provide background information to support the motivation for the study, and state the study objectives or hypotheses. Specifically,

- Provide context for the study, including information on the exposures and outcomes and why they are relevant to environmental health.
- Briefly review the literature to summarize current knowledge.

    - Present a balanced review of the literature, and acknowledge inconsistencies, rather than noting only findings that support the present study hypothesis.
    - For each cited study, indicate whether the research was observational or experimental, and note key characteristics of study populations or experimental models.
- Identify knowledge gaps addressed by the current study.
- Provide a clear description of the study questions/hypotheses, aims, or objectives, and, if appropriate, an overview of the approach used to address them.

Do not summarize study results or conclusions in the Introduction.

Methods

EHP requires complete methodological transparency—describe methods in enough detail to ensure that the study or analysis could be repeated by other researchers in the same field (at least in theory), and that the methods can be understood and interpreted by most *EHP* readers. Specifically,

- Thoroughly describe the methods used to generate all results reported in the manuscript, including (as appropriate):

Experimental studies	**Observational studies**

• Study design and experimental model
• Assay methods and conditions
• Justification of exposure and/or doses
• Number of biological and/or technical replicates
• Statistical analyses
• Accession numbers (or "rs" numbers for SNPs)
• All criteria used to interpret results
• Key assumptions and limitations of the methods
• Model numbers of all equipment used
• Company name, catalog number, and lot numbers for all reagents used
• Names/version numbers for data analysis software packages or macros
• All relevant details listed in the latest version of the ARRIVE guidelines
• Indication that the protocol was approved by an institutional animal care and use committee
	• Study design and population
	  ○ Report how and by whom "race" or "ethnicity" was defined (e.g., self-reported by participants or some other method) and why this information was included in the study design
	• Explain the rationale for treating race as an exposure, confounder, effect modifier, or other type of variable in analyses
	• Disaggregate race and ethnicity data to the fullest extent possible
	• Methods to measure or estimate exposures and covariates
	• Outcome definitions and ascertainment or measurement
	• Assay methods and conditions
	• Statistical analyses, including
	  ○ Statistical models and assumptions (with equations as appropriate)
	  ○ Methods/rationale for selecting model covariates (provide directed acyclic graphs as appropriate)
	  ○ Missing data methods
	  ○ Methods for assessing linearity/non-linearity
	  ○ Cutpoints for categorical variables
	• Sensitivity and secondary analyses
	• All criteria used to interpret results
	• Key assumptions and limitations of the methods
	• Names/version numbers for data analysis software packages or macros
	• Information about institutional review board approval

	• Describe informed consent protocols or explain why informed consent was not required

- If referring to previous publications for methods details, include a brief description of the approach, key assumptions and limitations, and any deviations from previously described methods.
- Do not report results in the Methods section unless relevant to explain the rationale for the approaches listed.

Results

All results on which study conclusions or inferences are based (in whole or in part), including null findings and results of secondary or sensitivity analyses, must be reported in full in the main text or in supplemental tables or figures (see "Supplemental Material" for a list of materials that may be presented in this section).

The "Results" section may be organized using subheadings that describe the nature of the results, but do not use declarative statements indicating your conclusions about the findings.

- Provide a clear and concise description of all findings without extrapolating beyond the study results. Interpretations of the findings should be reserved for the "Discussion" section.
- Do not describe methods for the first time in the "Results" section.
- Do not limit results to statistically significant results or selected findings that support the study hypothesis.
- In general, *EHP* recommends that authors avoid using statistical significance testing as the sole or primary criterion for interpreting their findings, but if significance testing or p-values are used, report numeric p-values (rounded to 1-2 significant digits) for all results instead of indicating whether results are above or below a specific p-value only.
- Clearly indicate the number of observations for each analysis or experiment. Numbers should reflect observations included in each analysis after accounting for missing data.
- Include an appropriate measure of precision or variation (e.g., standard errors, 95% confidence intervals) with all summary estimates and estimates of effect.
- For observational studies, include a table or tables summarizing relevant population characteristics, including all covariates included in primary or secondary models.
    - Indicate numbers of observations with missing data for all covariates.
    - Provide detailed information about exposure distributions, including minimum and maximum values, percentiles, and numbers of samples above/below assay limits of detection or quantification.

- Although *EHP* encourages the use of supplemental tables or figures for secondary findings (see "Supplemental Material" for details), present primary results in the main text. This includes results that are mentioned repeatedly, are related to the primary study aims, or are mentioned in the abstract or manuscript conclusions.
- Provide tables with corresponding numeric data for all figures (in the main text or supplemental material, as appropriate) or include numeric data within figures (e.g., as forest plots).

Discussion

Begin with a *brief* overview of the main study findings, without repeating all results in detail.

- Provide a review of the relevant literature and other information needed to put the study findings into context.
- Provide a complete and balanced view of previous research, including findings that are inconsistent with the hypothesis, results, or conclusions of the present study.
- Describe sources in sufficient detail to ensure that readers can assess the quality and extent of the contribution, including:
    - study type or design
    - sample size
    - population or experimental model
    - specific exposures and outcomes
- Provide a frank discussion of study limitations.
    - If race/ethnicity is included in analyses, discuss limitations in its classification, and in its use as a proxy for unmeasured consequences of racism.
- End with a summary of the key findings and their implications for the study question/hypothesis, future research, and policy, as appropriate.
- Do not describe methods or results for the first time in the "Discussion" section.

Acknowledgments

Include sources of funding for the research (if applicable), such as granting agencies, foundations, private support, etc. Authors may also include (as relevant) specific author contributions, acknowledgment of other contributors, information about data sharing, or names of large cohort groups.

Data Sharing

Information about data sharing protocols, options for accessing data, and links to data repositories may be provided in the “Acknowledgments” section, as noted above. Authors may also provide links to data repositories in the “Methods” or “Results” sections of their manuscripts, as appropriate. Genomics data

should be deposited into an acceptable repository (e.g., the National Center for Biotechnology Information) and made accessible to readers.

References and Citations

References

Begin the list of references on a new page after the "Discussion" section of the manuscript. Please provide complete, accurate information for references, including:

- Author/editor name(s) or authoring agency
- Year of publication
- Full title of article or chapter
- Title of journal or book/proceedings
- For books and meeting reports, city/state/country of publication and name of publisher
- Volume and inclusive page numbers
- PubMed article identifier (PMID)
- Digital object identifier (DOI)
- For websites and online documents, the URL and date accessed
- For software, the version number
- For data sets or data files, the electronic location or identifier, and version number or date accessed as appropriate

If you are uncertain whether to include a piece of information, err on the side of inclusion.

Number references numerically according to the order in which they first appear in the main text of the manuscript.

In-Text Citations

Place all in-text citations immediately after the information cited, using superscript numbers. Place citation numbers outside periods and commas but inside colons and semicolons, as shown below:

- High sodium intake has been strongly associated with increased risk of hypertension.[1] In some coastal areas, highly saline drinking water adds to people's sodium intake.[2]
- Other considerations in water safety include emerging chemicals of concern that cannot always be removed, such as pharmaceuticals[31,34] and nanoparticles[33]; aging infrastructure under streets and inside homes[34]; and other persistent threats, such as Legionella bacteria.[35–37]

When citing an electronic source in the reference list (website/web page/database), use a direct link to the specific report, document, or fact sheet where possible. References that direct readers to a generic homepage should be removed from the reference list and inserted as an in-text citation.

Reference Managers

EHP does not have dedicated reference manager style. If using reference manager software, select any established style that uses superscripted numbered citations, such as AMA.

Tables

EHP formats tables prior to publication. The editors reserve the right to request that complex tables be simplified to comply with Section 508 requirements.

Direct questions concerning tables to ehpsubmissions@niehs.nih.gov.

Creating Main Text Tables

- Begin each table on a new page after the list of references.
- Create tables using the Table feature in Microsoft Word. Do not submit tables as images.
- Number tables using Arabic numerals (e.g., Table 1, 2, 3, etc.) according to the order in which they are first mentioned in the main text.
    - Tables may not contain parts (e.g., Table 1A, 1B, etc.; or Table 1.1, 1.2, etc.).
- Ensure that all tables are cited in the main text.
- Give each table a title that describes what is shown but does not summarize results or present conclusions.
- Adhere to the following guidelines to ensure table readability for readers with disabilities:
    - Avoid using more than three layers of row or column headings.
    - Do not change column headings within the body of a table.
    - Do not merge cells across rows or across columns within the body of the table. All columns within the body of a table must comprise the same number of rows, and all rows must comprise the same number of columns.
    - Do not use shading, color, italics, underlining, or bold type for emphasis or to denote significance.
    - Do not include images or complex equations in tables.

Table Content

- Use the "±" symbol for arithmetic mean and standard deviation or standard error (e.g., "mean ± SE") and parentheses for the standard error when presented with the geometric mean [e.g., "GM (SE)"].
- Present number and percent as "*n* (%)" in one column.

- Present confidence intervals in parentheses in the same column as the point estimate, with the upper and lower bounds separated by a comma [e.g., (0.1, 2.3)].

Table Notes

- List abbreviations, definitions, and general information about the table in a note immediately under the table.
    - Define relevant populations or samples, models, calculations, variables, and statistical analyses such that the table can be interpreted easily by the reader without having to read the entire manuscript.
    - Indicate numbers of observations (overall and according to subgroups, as appropriate) used to derive the data shown, after accounting for missing data
    - If *p*-values are reported:
        - Indicate the comparison to which the *p*-value applies (e.g., "compared with untreated controls")
        - Indicate the statistical analysis used to derive the *p*-value
        - Provide numeric *p*-values for all estimates reported in the table, instead of using symbols to indicate *p*-value categories only
- List footnotes after the general note (if one is included) to explain or expand upon specific elements of the table.
    - Begin each footnote on a new line.
    - Indicate footnotes using lowercase italicized superscript letters, starting with "a" for each table. Lettered footnotes within the table should be ordered from top left to top right, next row left to right, and so on.
    - Do not use footnotes in the table title.

Figures

EHP does not redraw or format author images prior to publication. It is the authors' responsibility to ensure appropriate figure numbering, quality, and sizing to avoid publication delays.

EHP editors reserve the right to request that complex figures (e.g., figures with multiple panels showing information in a variety of formats, or that include panels related to different experiments) be divided into separate figures for publication. Authors also may be asked to edit figures to comply with Section 508 requirements.

Direct questions concerning figures to ehpsubmissions@niehs.nih.gov.

Creating Main Text Figures

- Number figures according to the order in which they are first mentioned in the main text.

- Ensure that all figures are cited in the main text.
- Adhere to the following guidelines to ensure figure readability and accessibility:
    - Do not use color as the only means of conveying information; use contrast, patterns, or symbols instead of color (or in addition to color) whenever possible.
    - Whenever possible, ensure that all images can still be interpreted when printed in black and white.
    - Ensure all words are spelled correctly.
- Clearly label all axes, giving both the measure and the unit of measurement where applicable.
- Ensure that letters, numbers, and lines are clearly legible and easy to differentiate and that all text within each image is of similar size, with type sizes at 6 point (minimum), though preferably at 8 points or above when reduced to final publication size.
- When possible, ensure that terms are styled the same in figures as they are in the main text (e.g., subscript the "10" in "PM_{10}" in both the text and the figure labels/legends).
- Ensure that terms and styles (including symbols and colors) are consistent across figures. For example, if Figure 1 is a scatterplot and Figure 2 is a bar graph, you might use a black circle to represent the control in the scatterplot and a black bar to represent controls in the bar graph.
- For photomicrographs, provide a scale bar on the image or report the original objective used to take the image. Do not adjust the magnification based on camera adaptor or eyepiece lenses. If a scale bar is provided, specify the length in the figure caption (e.g., "bar = 10 μm"). You may adjust an image for brightness and contrast if you apply the change to the entire image. Do not remove background data of gels and blots. The final image must accurately represent the original data.
- Graphs used to summarize data should include individual data points in addition to summary values or regression lines when possible.

Figure Size

- Figures may be no larger than 7.5 inches in width. Ensure that reducing a figure to this size does not compromise readability, quality, or interpretability.
- These guidelines also apply to figures with multiple panels. *EHP* does not have the ability to rearrange panels within a figure to meet the size requirement.

Saving and Submitting Figures

- Save and submit each main text figure as an individual file in one of the following formats:
    - PDF (fonts must be embedded)
    - PS/EPS (embed fonts, or use system fonts only: Helvetica, Courier, Arial, Times)

    - TIFF (no layers, LZW compression, Interleaved Pixel Order, IBM Byte Order, minimum 300 dpi, 600 dpi preferred, minimum 8-bit color depth)
    - JPG (may be submitted if higher-quality image formats are not available; minimum 300 dpi, 600 dpi preferred, minimum 8-bit color depth)
- Submit only one version of each figure, but format can vary by figure.
- Submit figures with multiple panels as a single file.
- Include the figure number in the filename of each figure (e.g., "Figure 1.pdf").
- Do not embed figures in the main text file.

Figure Captions

- Provide main text figure captions on a new page of the main text after tables.
- Include a title for the entire figure and descriptors for each panel [e.g., "Figure 1. Incidence of hepatocellular adenomas (*A*) and carcinomas (*B*) in mice exposed to DEHP"].
- Figure titles should describe the figure and not interpret its meaning or present conclusions.
- Define all uncommon abbreviations.
- Define relevant populations or samples, models, calculations, observations per data point, and statistical analyses such that the table can be interpreted easily by the reader without having to read the entire manuscript.
- Define all elements of the figure, including error bars, confidence intervals, symbols, whiskers, and lines or bars that are not already defined within the image itself.
- If statistical significance or *p*-values are reported, clearly indicate the comparison(s) to which they apply (e.g., "compared with controls from the corresponding age group").
- Provide a credit line for any images reused with permission from the copyright holder. Present credit lines as the copyright holder requires; do not reword.

Supplemental Material

Reserve Supplemental Material for background information that is needed to support transparency but not required to understand and interpret the primary findings. The main text must stand alone in the absence of Supplemental Material. Supplemental Material will be peer reviewed along with the manuscript and thus must meet the same rigorous standards. There is no limit on the number of tables or figures in Supplemental Material.

What Goes in Supplemental Material?

In general, Supplemental Material should be limited to results of secondary analyses and background details needed to ensure transparency, such as:

- Study questionnaires
- Lists of reagents and sources, SNPs, and primers
- Background data, such as lists of consortium members or detailed information on studies in systematic reviews
- Tables and figures with results of sensitivity analyses
- Tables with numeric data corresponding to results shown in the main text figures
- Directed acyclic graphs (DAGs) used to select model covariates
- Software code
- Raw data

What Does NOT Go in Supplemental Material?

- Text descriptions of study methods
- Tables or figures cited multiple times in the main manuscript, or that include results mentioned in the "Abstract" or "Discussion" sections
- Information or material that is not directly relevant to your study or manuscript

If you are uncertain about whether something should be included in the main text or in Supplemental Material, include it in the main text. If your manuscript is sent back to you for revisions, your editor will let you know if anything should be moved to Supplemental Material. *EHP* editors may decline to send new submissions out for peer review if methods are provided in Supplemental Material.

File Types

Supplemental Material (tables and figures) is usually submitted as a single Word file separate from the main text. However, content may be provided as separate files in alternative formats as appropriate:

- Excel files (ideal for large tables; see section below on "Preparing a Supplemental Excel File")
- Data analysis code and data files in appropriate formats for their intended use
- Video files (MP4 and AVI are preferred)
- Audio files (WAV or MP3 format)

Preparing Your Supplemental Material

Supplemental Material files are linked to their associated articles through a common DOI. Supplemental Material will be published as is without additional formatting or copyediting. Therefore, please confirm that your files are complete, accurate, and appropriately formatted for publication.

- Provide text (if necessary) first, followed by all supplemental tables, then all supplemental figures; do not alternate between figures and tables.

- Use descriptive headings to indicate information other than tables and figures, and refer to the headings when citing the material in the main text. For example:
    - see Supplemental Material, qRT-PCR primers
    - see Supplemental Material, Reagents
- When generating supplemental tables and figures:
    - Follow the formatting guidelines provided for main text tables and figures.
    - Provide the title for each figure below the figure on the same manuscript page as the figure itself.
    - Provide the title for each table above the table and the notes below the table.
    - Number supplemental tables and figures separately according to the order in which they are first mentioned in the main text. Use an "S" prefix with each table or figure number (e.g., Table S1, Figure S1).
    - Use landscape (i.e., horizontal) layout if necessary.
- Conclude the main Supplemental Material file with a list of references for any sources cited in the Supplemental Material, even if they are also cited in the main paper.

Preparing a Supplemental Excel File

Use Excel format only when it is not practical to include a table in the main Supplemental Material file (e.g., if it is too wide to fit on a single manuscript page or is more than two pages long).

- Provide multiple Excel tables in a single Excel workbook as separate worksheets.
- Label the tab for each worksheet with the indicator "Excel" and the table number (e.g., Excel Table S1). Number Excel tables separately from other supplemental tables.
- In the first row of the table, include the table number and title.
- Include a separate worksheet with explanatory information that applies to multiple tables as appropriate.
- Name the Excel file "Supplemental Excel File."