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Use of Urinary *p*-Nitrophenol as an Index of Exposure to Parathion

N. Denga,[1] P. Moldeus,[3] O. M. J. Kasilo,[2] C. F. B. Nhachi[1]

[1]Department of Clinical Pharmacology
[2](DaTIS) Department of Pharmacy, School of Medicine, University of Zimbabwe, P.O. Box A A178, Avondale, Harare, Zimbabwe
[3]Department of Toxicology, Karolinska Institute, Box 60400, S-10401 Stockholm, Sweden

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The organophosphorous (OP) insecticide parathion, (0,0-diethyl 0-4-nitrophenyl phosphorothioate) like many other OPs inhibits cholinesterase activity in most animal species, (Wallace and Kemp, 1991). Parathion is converted in vitro and vivo to paraoxon, the toxic oxygen analogue, (Neal, 1967a). Phosphorylation of acetylcholinesterase (AChEs), by paraoxon results in the release of p-nitrophenol. The release of the diethyl hydrogen phosphate from the esteratic site of the enzyme occurs extremely slowly hence a low turnover number of the enzymes, (Neal 1967b). This is indicative of the anti-cholinesterase activity of organophosphorous compounds. In Zimbabwe parathion is used as an insecticide mostly (but not exclusively) in coffee plantations in the Eastern Highlands region. Parathion poisoning is mostly through occupational exposure. With a large number of workers involved in the manufacturing, formulation and application of parathion, the risk of poisoning is high, thus providing the need to identify a toxicity index that can be rapidly used to detect and determine parathion exposure and the extend to which that occurs. The objective of this paper is to establish the use of urinary p-nitrophenol as a marker (index) of exposure to parathion in humans.

In this paper investigation and explanation of results obtained from an animal model (male rabbits), are used to demonstrate the possibility of using urinary p-nitrophenol, (a metabolite of parathion which is excreted in urine) as the exposure index of parathion compared to anti-cholinesterase activity of parathion in blood. In the absence of exposure to parathion, humans do not excrete p-nitrophenol. We suggest that use of p-nitrophenol is more acceptable in view of the obvious risks involved in handling human blood and the advantage of the non invasive use of urine.

METHODS AND MATERIALS

Urinary p-nitrophenol was determined using two spectrophotometric

Correspondence to: C. F. B. Nhachi

methods i.e. the method of Elliot et al, 1960 and the method of Moldeus et al, 1976. A chromatographic (GC) method was also employed (Bradway and Shafik, 1973). Male rabbits (3) of homogeneous strain (ZIKA), cross breed, of weight 2.85 $\pm$ 0.002 kg, raised from the institute's animal house were used. Before i.p. injection of parathion, in corn oil at 1.5 mg/kg body weight, venous blood samples were collected twice a day (8.30 am and 3.30 pm) from the ear veins of the rabbits, for five days. Each blood sample was fractionated into two i.e. plasma and red blood cells (RBC). Cholinesterase activity in each fraction was determined using the photometric method of de Huaerga et al, 1952. This method is based on the reaction of the test sample with alkali hydroxylamine after which the pH of the solution is restored by addition of 1N hydrochloric acid. Addition of ferric chloride develops the colour which is read at 540mu. Whole blood cholinesterase levels were also assessed using the WHO field kit spectrophotometer which is based on the method of Limperas and Ranta, (1953), as modified by Edson, (1955). Twenty four hour urine samples were also collected using metabolic cages. The collection of both samples was continued for periods of 5 to 6 days after i.p. injection of parathion. Both total serum protein, (Cornal et al 1949) and albumin (Doumus and Waston, 1971) were evaluated in all cases.

Parathion was obtained from Sulpeco,(Zimbabwe) at a purity of 99%. All the other chemicals were obtained from local, (Zimbabwe), chemical agencies and Sigma at a purity of 99%.

RESULTS AND DISCUSSION

In both methods used to assay for urinary p-nitrophenol, the presence of p-nitrophenol was not detectable in control animals. However p-nitrophenol levels in urine were detected 24 hours after parathion administration and peaked after 48 hours (table 1).

The results from table 1 suggest that the colorimetric method of Moldeus et al (in the evaluation of urinary p-nitrophenol) seem more sensitive than the Elliot et al method. It is also interesting to note that the depression in cholinesterase activities in rabbit, which occurred between 24 and 52 hours is not consistent. This is most likely due to the species dependent activity of cholinesterase.

Going by these results, we have opted to utilise the Moldeus method as an index of parathion exposure in humans. (It should be pointed out though that this method is not specific for RBC or ChE). To this end 21 coffee sprayers, (the men spray parathion in coffee plantations) were evaluated for possible exposure to parathion. The men were of average age, 36.5 $\pm$ 2.5 and had been spraying parathion four weeks prior to evaluation. In some of the cases, p-nitrophenol was detected even in those cases where the cholinesterase levels appeared normal, (table 2). This

perhaps points to the better sensitivity of the PNP method compared to cholinesterase levels.

Table 1. Cholinesterase activities and urinary pnitrophenol levels from 3 rabbits after i.p. parathion, (1.5 mg/kg body weight).

Time (Hours) Post admin.	Cholinesterase activities (UKat/L)		Urinary p-nitrophenol (µg/ml)	Protein content (g/100ml)
	[1]Total [2]AcChE [3]BuChe	ChE Activities %	*Method I **Method II	+Total Protein ++Albumin
0	7.392	100	0	5.00
	2.688	100	0	2.68
	4.704	100		
4	6.048	82	0	5.44
	7.392	275	0	2.36
	0.000	0		
24	4.032	55	2.284	4.64
	1.344	50	1.852	2.55
	2.688	57	-	-
28	1.344	18	-	5.76
	2.688	100		3.39
	0.000	0		
48	1.344	18	6.954	4.66
	0.784	29	5.093	2.11
	0.656	14		
52	2.016	27	3.854	5.06
	1.344	50	2.105	2.71
	0.672	14		
72	6.720	91	1.772	5.10
	7.392	275	2.050	2.85
	0.000	0		
144	4.704	64	0.402	5.55
	0.000	0	1.521	3.03
	4.704	100		

[1]Total ChE indicates whole blood cholinesterase activity, [2]AcChe indicates plasma cholinesterase activity and [3]BuChE indicates red blood cholinesterase. *Method I indicates the adopted method of Elliot et al(1960) and **Method II indicates the method of Moldeus et al.(1976). Protein levels were measured as +Total protein and ++Albumin.

Table 2. Cholinesterase activities and urinary p-nitrophenol levels in parathionsprayers.

Number of Sprayer	%ChE (whole Blood)	ChE Activity (Ukat/L)	Urinary p-nitrophenol µg/ml
1	<100	30.240 30.240 0.000	1.587
2	<100	48.384 34.272 14.112	4.44
3	<100	52.416 32.256 20.160	6.35
4	<100	46.368 36.288 10.080	3.49
5	<100	42.336 32.256 10.080	5.20
6	<870	40.320 26.208 14.112	8.571
7	87.5	54.432 36.288 18.144	4.44
8	<100	46.368 22.176 24.192	4.13
9	<100	50.400 38.304 12.096	3.81
10	75-87.5	61.152 30.240 30.912	3.49
11	<100	50.400 30.240 20.160	0
12	<100	59.136 30.240 28.896	0

Table 2. continued

13	100	38.304 16.128 22.176	0
14	37.5	58.464 26.208 32.256	7.62
15	87.5	54.432 24.192 30.240	0
16	87.5- 100	46.368 18.144 28.224	0
17	87.5	44.352 34.272 10.080	0
18	75	46.368 24.192 22.176	0
19	<100	56.448 34.272 22.176	0
20	75	66.528 36.288 30.240	0
21	<100	78.624 48.384 30.240	0

*The blood protein levels, (total and albumin), were very constant with an average mean value of 6.71 $\pm$ 1.83 and 1.80 $\pm$ 0.30 respectively. The p-nitrophenol levels in table 2 are calculated as shown in table 3. The data in table 2 indicates that urinary p-nitrophenol may be used a biological marker of exposure to parathion in humans but not necessarily of the extent of exposure. This is because exposure to parathion is revealed by the presence of urinary p-nitrophenol even in those individuals whose cholinesterase activity (using the WHO field kit method), appeared normal. Perhaps the reason why the workers showed low levels is because their protective clothing was adequate. For protective clothing the workers put on overalls, boots, gloves and face masks. In addition use of urinary p-nitrophenol as an index of exposure to parathion offers the advantages of avoiding

invasive methods with the added attraction of simplicity elegance and appreciable sensitivity.

Table 3. Estimation of absorbed dose of parathion from urinary p-nitrophenol levels.

Worker	Urinary p-nitrophenol (PNP) (µg/ml)	(mg/day)*	(µmoles**)	Parathion absorbed mg/day ***	mg/kg/day *****
1	1.59	2.38	11	3.32	0.05
2	4.44	6.66	32	9.30	0.13
3	6.35	9.53	46	13.29	0.19
4	3.49	5.24	25	7.31	0.10
5	5.20	7.80	37	10.89	0.16
6	8.57	12.87	62	17.94	0.26
7	4.44	6.67	32	9.30	0.13
8	4.13	6.19	30	8.64	0.12
9	3.81	5.71	27	7.97	0.11
10	3.49	5.24	25	7.31	0.10
11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
14	7.62	11.43	55	15.95	0.23
15	0	0	0	0	0
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	0	0	0
19	0	0	0	0	0
20	0	0	0	0	0
21	0	0	0	0	0

* assume 1500 ml/day; 70kg human
** µmoles PNP/day = mg/day x 0.001 = g/day/MW PNP (139) x 10^6
*** mg/parathion/day = moles PNP x MW parathion x 100
**** mg/kg/day = mg parathion/day/70kg

Exposure (mg/kg/day): = 0.14 (mean); = 0.08 (standard deviation)

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