SUB-CHRONIC ADVERSE EFFECT OF PARATHION ON MALE ALBINO RAT (*RATTUS NORVEGICUS*)

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Abstract

The present work is an attempt to confirm the sub-chronic adverse effects of parathion, either in its technical (99 % ai) or formulated form (40 % EC) on blood picture, liver function, cholinesterase activity and cytochrome-C- system in male albino rat (*Rattus norvegicus*). Animals received a sub-lethal dose (1/20 LD50) of technical or formulated parathion either orally or dermally at 2-day intervals for 90 days on the light of the protocol proposed by EPA (1979).

Results revealed that parathion insecticide caused signficant oligocythemia accompanied by severe haemoglobinaemia resulting in significant rise in total bilirubin level in plasma which confirm its haemolytic effect. The hepatic toxicity of parathion was evident through the hyperactivation of transaminases indicating a hepatic disorder and/or damage. On the other hand, a significant decline in the plasma cholinesterase activity was recorded confirming the neurotoxicity of this organophosphorus insecticide. Furthermore, the respiratory toxicity of parathion was ascertained. The treatment caused 6-23% fall in the cytochrome-C content of different tissues showing a destructive effect to mitochondria. Contrarily, cytochrome-C oxidase and succinate cytochrome C-reductase activity was enhanced which appeared to be due to the increase in the dehydrogenases activity rather than the stimulation of respiratory system.

The present results also indicated that parathion revealed haematohepato, respiratory as well as neuro-toxicity even when administered at sub-lethal doses. In addition, the formulated form exhibited higher toxicity than the technical one indicating that the additives must have possessed an inducing particularly when given orally. This leads to the confirmation that the final toxic classification of pesticides must be based on both the technical and formulated forms.

INTRODUCTION

Pesticides may be continually adminstered over long periods. Thus, causing chronic toxicity, a matter that threatens the environment .

Pesticides are usually applied in their formulated forms where the active ingredient is combined with organic solvents, emulsifying and wetting agents, which affect the pesticide penetration. In addition, these additives may cause synergism or antagonism to the toxicity of the active ingredient (E1-Sebae, 1985). Consequently, the W.H.O. (1991) emphasized that the final toxic classification of any pesticide is intended to be by its formulation.

Since active ingredient and formulated pesticides differ in toxicity to mammals, there is a need to investigate and compare their metabolic and toxicological properties (Daves *et al.*, 1992) .

Many toxicological studies were carried out on parathion resulting in the pesticide being banned. Recently, Abdel-Rahim and Eweis (1995), for instance, observed that formulated parathion reduced the body weight gain much more than the active ingredient, but elevated blood sugar and stimulated the thyroid gland. Also, Abdel-Rahim *et al*, (1995) recorded that tissue ATP content increased and the activity of acid and alkaline phosphatase was stimulated by parathion treatment and that the formulated form was more effective than the technical one .

The present work is an attempt to confirm the biochemical damage caused by parathion taking in consideration both the technical and formulated form. The effect of the pesticide on blood hemoglobin (Hb) content, red blood cells (RBCs) and white blood cells (WBCs) count, plasma total bilirubin, plasma and liver total soluble protein, activity of plasma and liver GOT and GPT as well as the liver, brain and kidney's cytochrome-C. content and the activity of cytochrome - C - oxidase (1.9.3.1) and succinate cytochrome-C-reductase (1.3.99.1) in adult male albino rat (*Rattus norvegicus*).

MATERIALS AND METHODS

1- Chemical used

Parathion (O,O diethyl-O-P-nitrophenyl phosphorothionate) either pure (99% ai) or formulated (40 % E.C.) was a gift from ICI plant protection Division, Giza, Egypt and was used throughout the present study.

2- Animals

Fifty healthy adult male albino rat (Rattus n.) were withdrawn from the animal house of the Department of Biochemistry, Faculty of Agriculture, Cairo University. Throughout the acclimatization period (2 weeks) and the experimental period (90 days) animals were kept under normal health conditions and provided with suitable accommodation. (Lane . Peter and Pearson, 1971).

3- Experiments

Animals were divided into five groups (10 animals each). The first group was maintained as a control one while the others were treated with pure or formulated parathion either orally or dermally. Each animal received a sub-lethal dose of 1/20 LD 50 of parathion at 2 day interval for 90 days on the light of the protocol proposed by EPA (1979). At the end of the experiment whole blood samples were collected from the orbital sinus by eye puncture then animals were killed, dissected and liver, brain and kideny were obtained.

4- Assays

Tissue homogenate was made up in a saline solution. Mitochondria was emulsified with 1% triton X-100 (3 ml) at 0°C for 30 minutes according to Astawrov (1974). Mitochondrial enzymes were liberated and assayed. Cytochrome-C Content was determined according to the method of Williams and Thorp (1969). Cytochrome C-oxidase activity was determined according to Smith (1955) and succinate-cytochrome-C-reductase activity was assayed as described by King (1963). Plasma total bilirubin was estimated as demonstrated by Jendrassik and Graf (1953). Total soluble protein was determined by the method originated by Bradford (1976). Cholinesterase activity was determined according to the method of Eliman *et al.* (1960). Glutamate-oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were determined as shown by Reitman and Frankel (1957). Total hemoglobin, red blood cells (RBCs) and white blood cells (WBCs) counts were determined according to Dacie and Lewis (1975). Data were subjected to t-test as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

1- Effect of the insecticide on blood picture

Data in Table 1 show that the administration of parathion, either orally or

dermally resulted in significant decrease in the erythrocytic count referred to as oligocythemia which was associated with a parallel decrease in Hb content leading to the haemoglobinaemia. Contrarily, the insecticide effect on the leukocytic count was insignificant i.e. the body maintained a normal count of WBCs. These results agree with those of Kuntz *et al.* (1990) and Abdel-Rahim *et al.* (1994).

The observed influence upon blood picture has several causes, i.e. the malignant tumours of animal organs, particularly the liver, which is combined, to a great extent to the chronic exposure to a chemical (Sood, 1990). The oligocythemia could be attributed to the destructive effect of the insecticide on either the haematopoietic system, thus leading to the failure to provide the circulation with proper count of RBCs or even on mature erythrocytes themselves resulting in haemolytic anaemia. The overall effect of the pesticide was confirmed with the formulated form particularly by the oral route. This influence seems to be more agreeable if we take into account the effect of the insecticide together with the associated ingredients as well as the enhanced bioavailability of the pesticide in case of the oral route .

Table 1. Influence of technical and formulated parathion on total haemoglobin content and RBCs and WBCs count of blood.

Treatment	Total haemoglobin g/dL blood	%	RBCs count count x 106	%	WBCs count count x 103	%
Control	15.00 ± 1.11	100	8.63±1.00	100	6.40±0.59	100
Oral						
Technical	12.20*±1.32	81	7.00±0.63	81	6.32±0.55	99
Formulated	11.00*±1.01	73	5.71*±0.60	66	6.11±0.62	95
Dermal						
Technical	14.10±1.32	94	7.87*±0.80	91	6.39±0.64	100
Formulated	1.41*±1.21	83	7.10*±0.71	82	6.20±0.59	97

2- Effect of the insecticide on liver function

Data in Table 2 show that parathion resulted in severe rise in the level of total bilirubin in plasma i.e., Hyperbilirubinaemia ranged from 155% to 222% as much as the control. As is known, the erythrocytes when haemolysed, the released contents including haemoglobin are catabolized by the reticuloendothelial cells in the spleen,

liver and bone marrow and this biodegradation process results in several metabolites including bilirubin, easily detected in the blood and urine. Meanwhile, parallel significant rise in the protein level was recorded in both the plasma and liver. This rise ranged from 109 to 121% and from 128 to 153% as much as control, respectively and could be attributed to the stimulation of protein biosynthesis through various pathways (Rothman *et al.* 1990; Abdel-Rahim and Eewis 1995; Abdel-Rahim *et al.* 1995).

Table 2. Influence of technical and formulated parathion on total bilirubin and plasma liver soluble protein,

Treatment	Total bilirubin mg/100 ml	%	Protein content Plasma g/100 ml	%	Liver g/100 mg	%
Control	18.91 ± 0.10	100	7.01±0.67	100	19.91±1.11	100
Oral						
Technical	1.81*±0.20	199	8.00*±0.69	114	26.66*±2.37	134
Formulated	2.02*±0.20	222	8.51*±0.74	121	30.51*±2.94	153
Dermal						
Technical	1.41*±0.12	155	7.61*±0.73	109	25.56*±2.47	128
Formulated	1.77*±0.15	195	7.89*±0.80	113	27.11*±2.80	136

- Values are represented as mean ± SD
- Control Value is referred to as 100 %
- (*) Significant at $P<0.05$

Data in Table (3) confirmed the harmful effect of the insecticide on the liver. Administration of parathion resulted in significant rise in transaminases activity which indicates a hepatic disorder and/or damage. Similar findings were recorded for malathion by Abdel-Rahim *et al* (1994).

3- Anti-cholinesterase activity of parathion

A significant decline in plasma cholinesterase activity resulted from parathion treatment and this decline was more pronounced with the formulated form indicating a synergistic effect of the adjuvants (Table 3). This pronounced effect may be due to the direct effect of such additives on the nervous system i.e., having a neurotoxic effect or more probably due to the interference with any intermediate reaction/

process. The effect of detergents on chemical and biochemical reactions is well known. The oral route of administration was more effective, but the period of experiment was long enough to induce the dermal toxicity which is normally high with parathion. Similar results were shown for malathion (Abou-Zeid *et al.*, 1993). It is worth mentioning that parathion, among others reacts with cholinesterase in a manner analogous to that of the normal substrate and the resultant excess of acetyl choline at the neuromuscular junction can act as a blocking agent, depolarizing the motor and plate (Comben and Derache, 1980).

4-Effect of the insecticide on respiratory cytochrome-C system

The level of cytochrome-C in tissues decreased to about 77-94% of control (Table 4). Data suggest that cytochrome-C destruction occurred predominantly during the introduction of parathion. Cytochrome-C is synthesized extra-mitrochondrially to the cytosol ribosomes and is attached to the inner mitochondrial membrane (Schnaitmain and Greenawalt, 1968 and Lehninger *et al*, 1993). It could therefore serve as a good marker for inner mitochondrial membrane turnover. The present results strongly support the hypothesis that the period of the experiment was long enough to lead to the destruction of 6-23% of mitochondria.

The effect of parathion on the mitochondrial respiratory enzyme activity was investigated and the results are shown in tables (5,6). Parathion stimulated the activity of cytochrome-C-oxidase and succinate cytochrome-C-reductase. The increase was not due to an overall enhancement of activity of the respiratory system and appeared to be due to the increase in the dehydrogenases activity, the rate-limiting step of the oxidation of metabolites like succinate. This was further substantiated by the observed increase in the activity of succinate-cytochrome - C-reductase which includes the rate-limiting step catalyzed by the primary dehydrogenase and the limited stimulation of the activity of cytochrome-C-oxidase which is known to be far in excess of the overall rate of oxidation of the metabolite succinate (Lehninger, 1982).

The results in tables (5,6) showed that stimulation in the basal enzymes activity could be discerned in parathion given animals.

The great stimulation in the enzyme activity was reached by the oral treatment formulated parthion confirmed the effect of associate ingredients particularly the detergent. Stimulation in cellular oxidation as well as in the activities of several oxidoreductases by pesticides had been reported in animal tissue (Berberian, 1986

Table 3. Influence of technical and formulated parathion on plasma cholinesterase and plasma and liver GOT and GPT activities.

Treatment	GOT				GPT				Plasma Ch.E.	
	Plasma U/ml	%	Liver U/ml	%	Plasma U/ml	%	Liver U/ml	%	g/100 ml	%
Control	30.01 ± 2.99	100	10.0 ±	100	12.81 ± 1.30	100	40 ± 4	100	99 ± 10	100
Oral										
Technical	45.50*±4.41	152	156*±16	156	16.99*±1.71	133	55*±5	138	41*±4	41
Formulated	61.11*±5.91	204	200*±21	200	20.21*±1.91	158	72*±6	180	35*±3	35
Dermal										
Technical	42.16**±4.12	141	133*±13	133	15.01*±1.46	117	51*±5	128	51*±4	52
Formulated	44.21*±4.21	147	154*±14	154	16.44*±1.57	128	56*±6	140	40*±3	40

- Values are represented as mean ± SD
- Control Value is referred to as 100 %
- (*) Significant at P<0.05

Table 4. Influence of technical and formulated parathion on cytochrome-C-content of liver, brain and kidney.

Treatment	Liver Umole/g	%	Brain Umole/g	%	Kidney Umole/g	%
Control	23.24* ± 2.01	100	9.11±1.00	100	27.13±2.33	100
Oral						
Technical	21.24*±2.01	91	8.00*±0.79	88	24.14*±2.22	89
Formulated	20.12*±2.01	87	7.00*±0.66	77	22.12*±2.41	82
Dermal						
Technical	21.89*±1.92	94	8.11*±0.80	89	25.51*±2.41	94
Formulated	21.19*±2.21	91	7.93*±0.80	87	24.24*±2.33	89

Table 5. Influence of technical and formulated parathion on cytochrome-C-content of liver, brain and kidney.

Treatment	Liver Umole/g	%	Brain Umole/g	%	Kidney Umole/g	%
Control	15.97* ± 1.60	100	50.11±5.00	100	14.10±1.31	100
Oral						
Technical	17.66*±1.66	111	56.90*±5.17	114	15.71*±1.49	111
Formulated	18.12*±1.77	113	60.20*±6.00	120	16.02*±1.51	114
Dermal						
Technical	17.01±1.67	107	54.91*±5.32	110	15.00*±1.46	106
Formulated	17.59*±1.43	110	57.11*±4.17	114	15.40*±1.43	109

- Values are represented as mean ± SD
- Control Value is referred to as 100 %
- (*) Significant at $P<0.05$

and Abdel-Rahim *et al.*, 1994).

The present study showed that oxidative enzymes could increase the overall rate of oxidation of metabolites as succinate and represent a compensatory mechanism which overcomes the initial lack of 02 which provides the animal with energy requirement. Thus, the specific increase in the activity of oxidative enzymes in the treated rat organ could be due to the animal physiological status (Lehninger, 1982). The formulations may cause synergism to the toxicity of the active ingredient. Such formulations are expected to affect the parathion penetration, distribution and retention through the body tissues and blood constituents .

Table 6. Influence of technical and formulated parathion on succinate-cytochrome-C-reductase activity in liver, brain and kidney.

Treatment	Liver		Brain		Kidney	
	Umol/min./g	%	Umol/min./g	%	Umol/min./g	%
Control	2.01 ± 0.21	100	29.87±2.71	100	3.20±0.29	100
Oral						
Technical	3.80*±0.40	189	56.11*±5.47	188	4.41*±0.37	138
Formulated	4.00*±0.37	199	60.00*±6.10	201	4.80*±0.42	
Dermal						
Technical	3.54*±0.34	176	57.87*±5.79	194	4.21*±0.41	132
Formulated	3.77*±0.29	188	55.80*±5.21	187	4.32*±0.43	135

- Values are represented as mean ± SD
- Control Value is referred to as 100 %
- (*) Significant at P<0.05

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التأثير تحت المزمن الضار لمبيد الباراثيون على ذكور الفئران الألبينو

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الدراسة الحالية بمثابة محاولة لمعرفة الاثار الضارة تحت المزمنة لمبيد الباراثيون فى صورته النقية (٩٩ ٪ مادة فعالة) أو فى صورة المستحضر المعد للتطبيق (٤٠٪ مركز قابل للأستحلاب) وذلك على صورة الدم ووظيفة الكبد ونشاط أنزيم الكولين أستريز وكذلك نظام سيتوكروم وذلك فى ذكور الفئران الألبينو. عوملت الحيوانات بجرعة تساوى ٢٠ / ١ من قيمة الجرعة السامة النصفية (LD50) للمبيد من الصورة النقية أو المستحضر اما عن طريق الفم أو عن طريق الجلد وذلك كل يومين لمدة ٩٠ يوما.

أظهرت النتائج أن المبيد تسبب فى خفض كرات الدم الحمراء بصورة معنوية مقارنا بالحيوانات غير المعاملة – أدى هذا الأنخفاض الى أنخفاض موازى فى محتوى الدم من صبغة الهيموجلوبين ودلت الزيادة المقابلة فى محتوى البلازما من الصفراء أن المعاملة أدت الى حدوث تحطم الخلايا الحمراء وتسرب محتوياتها وحدوث هدم لهذه المحتويات. من ناحية أخرى تأثرت وظيفة الكبد بدرجة كبيرة حيث أزداد نشاط أنزيمات نقل الآمين بما يعكس حدوث تلف أو خلل فى أنسجة الكبد. كذلك – وكما هو متوقع – أدت المعاملة الى انخفاض نشاط انزيم الكولين أستريز فى بلازما الدم بما يؤكد السمية العصبية لهذا المبيد حتى عند أستخدام جرعات دون الجرعات القاتلة بالأضافة الى كل ما سبق أظهرت المعاملة بالمبيد بصورتية سمية تنفسية بصورة معنوية حيث أنخفض محتوى الأنسجة من سيتوكروم وكذلك أرتفع نشاط أنزيمى سيتوكروم (C) أوكسيديز وسكسينات سيتوكروم (C) رودكتيز . من المرجح أن هذه الزيادة فى نشاط الأنزيمين يعزى الى زيادة نشاط الديهيدروجينز (Dehydrogenases) وليس الى تنشيط نظام السيتوكروم. أظهرت الدراسة كذلك أن أثر المبيد أزداد فى حالة المعاملة بالمستحضر عنه فى حالة أستخدام المبيد النقى بما يعنى أن المواد الأضافية الموجودة بالمستحضر أحدثت تنشيطا لأثر المبيد على العمليات الحيوية المختلفة بالجسم سيما عند أعطاء المبيد عن طريق الفم. الدراسة الحالية أكدت أثر المبيد كسم عصبى وكذلك أثره غير المرغوب فى مكونات الدم ووظيفة الكبد وكذلك سميته التنفسية حتى عند أعطائه للحيوانات بجرعات صغيرة.