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PHARMACOKINETICS AND EXCRETION OF AMETRYN IN RAT FOLLOWING A SINGLE ORAL ADMINISTRATION.

BY

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ABSTRACT

Pharmacokinetics and excretion of ametryn (2-ethylamino-4-isopropyl amino -6-methylthio-s- triazine) following a single oral dose of 10 mg/kg were investigated in female rats. Animals were killed at time intervals of 15min 1, 6, 12, 24, 48, 72, 96 hr, and 7 days. On the basis of gram tissue, brain concentration of ametryn was higher than in plasma at the first time interval. The values were 0.40 and 0.07 ug/g(ml) which accounted for .07 and .01% of the applied dose, respectively. The daily rate of elimination in the excrements was found to increase slowly with time until two days after dosing. Following this, there was a slower rate of excretion of the herbicide. Approximately 4.2% of the dose was eliminated in the excreta throughout the seven days experiment. The concentrations of residual ametryn in both brain and plasma tissues declined exponentially by time. The half-life values of the elimination of ametryn from brain and plasma were 3.58 and 4.0 days corresponding to the rate constant values of 0.19 and 0.17 day-1, respectively. Brain AUC (area under the curve) value was 1680 mg.hr / kg, indicating that there was no tendency for the compound to be retained in the plasma (AUC = 240 mg. hr/l) compared with the brain.

INTRODUCTION

Agricultural pesticides are extensively used in Egypt. There is an increased concern about the adverse effects resulting from pesticide residues. Ametryn, a member of the s-triazine family of herbicides which has a selective role for control of broadleaf and grass weeds in various crops. Since it has a considerable contact activity, it is widely used in the preharvest desiccation of various crops, for post emergent and for aquatic weed control. It has low mammalian toxicity; oral LD50 values for mice and rats are 965 and 1100 mg/kg, respectively (Anonymous, 1983 and 1984).

In spite of many studies concerning the metabolism and excretion of 2-methylthio-s-triazine herbicides, e.g. ametryn, cyanatryn, dimethametryn and simetryn in mammals, plants, and fish which have been reported by Oliver et al, 1969; Crawford et al, 1980; Donzel et al, 1987; and Tsudaet al, 1989, there does not appear to be any information regarding the pharmacokinetics of ametryn. Series of our studies were conducted to examine the pharmacokinetic profile of pesticides in rat (Salamaet al, 1992a, 1992b, and 1992c) and mouse (Salama, 1992). Therefore this study was designed to provide the pharmacokinetic profile of ametryn in female rat following a single oral administration.

MATERIALS AND METHODS

Animals

Female white rats, Rattus norvegicus albinus, weighing 55 ± 2.4 g were obtained from the permenant colony maintained at High Institute of Public Health, Alexandria University, Alex.

Egypt. The animals were housed in transparent plastic cages covered by stainless steel wire lids. Rats were acclimatized in the laboratory and fed on a commercial standard rat diet ad libitum.

Chemicals

A technical grade sample of ametryn [N-ethyl-N-(1-methylethyl) -6-(methylthio) -1,3,5- triazine-2,4-diamine] (99.3% purity) was supplied by Ciba Geigy Co. All other chemicals were of the highest purity grade available from BDH Co., England.

Administration of ametryn

Fourty female rats were used for the study. A single oral dose of 10 mg ametryn /kg body weight in 1ml corn oil was administered. Animals were anesthetized and killed by heart puncture after the following time intervals: 15 min, 1,6,12,24,48,72,96 hrs, and 7 days.

Collection of excreta and tissues

Following administration, each animal was housed in a metabolism cage from which its urine and feces could be collected daily. At each time interval, four animals were anesthetized with diethyl ether and dissected. The blood was collected via cardiac puncture with heparinized syringes. Red blood cells were separated from the plasma by centrifugation at 2400 rpm for 5 min at 4 °C. Brain was dissected out rapidly, weighed, placed in glass vials, and stored at -20 °C until analysis.

Extraction of samples

All samples from each time point were extracted according to the method described by the Food and Drug Administration (Anonymous, 1987) with minor modifications.

Brain:

Brain samples were homogenized in acetonitrile (1:25 w/v). The homogenate was filtered and partitioned with dichloromethane (3x15 ml). The combined organic layers were desiccated over anhydrous sodium sulfate (2g).The extract was concentrated by rotary evaporator at 40 °C to near dryness and adjusted to 3 ml for column chromatography. Interfering materials were removed from the brain extract by passing it through disposable Pasteur pipets (14.6 x 0.75 cm id.) packed with florisil-alumina (1:1 w/w). The aliquots (3ml) were applied to the columns and eluted with 3 x 10 ml of dichloromethane. The eluate was concentrated as mentioned above and adjusted to 1ml prior to gas chromatographic analysis.

Plasma:

Plasma (0.5 ml) was diluted to 1ml with distilled water and extracted four times with 10ml of dichloromethane. The organic layers were combined, dried, concentrated as described in the case of brain. The clean up was carried out using alumina column (2g). The cleaned samples were evaporated and then subjected to gas chromatographic analysis.

Urine:

Urine, collected daily from the rats that were kept for 7 days after treatment, was extracted three times with 30 ml of petroleum ether and the combined extracts were dried, concentrated and passed through an alumina column, prior to analysis.

Feces:

Dried fecal samples were mixed with petroleum ether (1:20 w/v). The mixture was filtered, dried and concentrated to 1ml. Clean up was made using charcoal-alumina column (1:1 w/w) and eluted with 3x10 ml of petroleum ether. The eluate was concentrated to 1ml prior to GC analysis.

Fortification

Recovery percentages of ametryn from brain, plasma, urine and feces were carried out by the addition of ametryn to each at three levels of 0.1, 0.5, and 1.0 ug/g(ml). The fortified samples were analyzed as described before.

Gas Liquid Chromatographic Analysis

Analysis was performed on a Shimadzu-4CM (PFE) dual column GC, equipped with flame photometric detector (FPD) for sulphur mode according to Ramsteiner et al.1974, and an analytical glass column (2mx4mm i.d.) packed with 5% SE-30 coated on Chromosorb-W, 60-80 mesh. Operating temperatures (°C) were: column 230 isothermal, injector 230, and detector 250; gas flow rates (ml/min) were: nitrogen 40, hydrogen 80, and air 100. The limit of detection was 80 pg. Identification of ametryn was accomplished by retention time and compared with an authentic standard under the same conditions. The quantities were calculated on peak height basis, except for broad peaks which were calculated from area under the peak.

Pharmacokinetic modelling

For pharmacokinetic modelling of the parent herbicide tissue concentrations, an equation describing a one-compartment open model with first order input, no lag time and first order elimination from the central compartment was used (Gillette, 1974). The data were considered consistent with a monoexponential function [Ft = Ao exp -ket] where Ft is the fraction of dose in the body at time t, A is the central compartment of a one-compartment system, and ke is the disposition rate costants. Estimates of half-lives of ametryn in tissues (t1/2) and elimination rate from the central compartment were made by linear regression of the terminal linear exponential decline in ametryn concentration using the relationship t1/2 = 0.693/kel. The area under the tissue concentration:time curve (AUC) was calculated by the trapezoidal rule and extrapolated to infinity by using the last point and respective terminal linear exponential decline.

Statistical analysis

Analysis of variance (ANOVA) was used to compare means among treatments. The least square means were compared for significant differences between treatments ($P \leq 0.05$).

RESULTS AND DISCUSSION

Recovery of ametryn

Ametryn was extracted from brain, plasma, and excreta as described before. Recoveries of ametryn from tissues and excreta at three different levels of fortification are presented in table 1. The average recovery percentages were 89.85, 99.97, 92.65, and

103.89 % for plasma, brain, feces, and urine, respectively.

TABLE 1

Average percent recoveries of ametryn from fortified tissues and body fluids of female rats.

Added ug	% Recovery* Plasma	Brain	Feces	Urine
0.1	95.05 ±2.9	116.7 ±0.0	106.37 ±30.4	118.34 ±1.2
0.5	61.5 ±9.5	99.6 ±14.1	86.29 ±15.8	95.00 ±3.6
1.0	113.0 ±7.0	83.6 ±17.6	85.28 ±18.7	98.34 ±1.2
Average	89.85	99.97	92.65	103.89

* Each value represents the mean for four replicates ± S.E.

Brain and plasma uptake of ametryn

Ametryn was found in the brain and plasma sampled at 15 min, 1hr, 6, 12, 24, 48, 72, 96, and 7 days after a single oral dose of

10 mg/kg of the compound. The concentrations of ametryn after dosing are shown in table 2. Brain contained higher concentration of ametryn per gram at 15 min than plasma. The values were 0.4 and 0.07 ug/g (ml) which accounted for 0.07 and 0.01% of the applied dose, respectively. The concentrations of ametryn in plasma remained almost unchanged between the 24-48 hrs time points. This is probably due to the continuous absorption of ametryn from the intestine. The concentrations of residual ametryn then declined and by 7 days reached 0.19 and 0.01 ug/g(ml) in brain and plasma which accounted for 0.03 and 0.01% of the applied dose, respectively. The results illustrated in table 2, also indicate that ametryn level was higher in the brain than that in the plasma at all times after intubation.

In general, the distribution of the material after dosing was rapid with levels peaking within 12 hr in the plasma and 24 hr in the brain. This finding suggests rapid absorption of the compound from the intestine. Apparently, the compound was distributed in the different tissues via the blood soon after application, then metabolism was taking place and the amount per g. tissue dropped to a minimum 7 days after treatment.

By comparing the concentration of ametryn at various times after administration, it was possible to determine which of these organs had a greatest affinity to the compound (Matthews, 1979). Maximum ametryn levels in brain exceeded that in plasma by 3.5-19.0 fold. This finding suggested that brain tissue had a greater affinity for ametryn than for plasma components.

TABLE 2

Concentration of ametryn in brain and plasma of female rats after a single oral dose of 10 mg/kg.

Time hr	Plasma* ug/ml	% of dose	Brain tissue ug/g	% of dose	Brain/ plasma
15 min	0.07ab±0.01	0.01	0.40c ±0.17	0.07	5.71
1 hr	0.08ab±0.02	0.02	0.28d ±0.07	0.06	3.50
6	0.12a ±0.02	0.03	0.22e ±0.03	0.05	8.33
12	0.15a ±0.03	0.03	0.56ab±0.09	0.11	3.73
24	0.13a ±0.03	0.02	0.60a ±0.10	0.10	4.62
48	0.13a ±0.02	0.02	0.53b ±0.00	0.09	4.08
72	0.10a ±0.00	0.02	0.38c ±0.03	0.07	3.80
96	0.08ab±0.03	0.02	0.31d ±0.04	0.07	3.88
7 day	0.01b ±0.00	0.01	0.19e ±0.06	0.03	19.00
LSD	0.009		0.042		

*Plasma values are calculated as follows: Blood vol. (6-7% of b.w) and packed cell vol. (46%).
Each value represents the mean for 4 samples + S.E
Means followed by the same letters are not significantly different at P < 0.05.

Urinary and fecal excretion of ametryn

Following the oral administration of ametryn, 0.58% of the applied dose was eliminated in the combined urinary-fecal excretion (table 3). The daily rate of elimination increased slowly with time until 2 days after the administration. Following this, there was a slower rate of excretion of the pesticide. Approximately 4.20% of the dose was discharged in the excreta throughout the

TABLE 3

Cumulative excretion of ametryn into urine and feces of female rats given a single oral dose of 10 mg/kg.

Time (days)	Cumulative amounts urine ug	urine % of dose	feces ug	feces % of dose
1	3.08g±0.26	0.47	0.67g±0.08	0.11
2	5.52f±1.50	0.85	1.34f±0.08	0.23
3	9.41e±1.90	1.45	1.66e±0.09	0.28
4	13.62d±2.20	2.10	2.18d±0.08	0.37
5	17.71c±4.60	2.73	2.61c±0.07	0.43
6	20.27b±4.10	3.12	3.04b±0.05	0.50
7	23.67a±4.50	3.65	3.42a±0.06	0.55
LSD	1.29		0.003	

Each value represents the mean for 4 samples ±S.E. Means followed by the same letters are not significantly different at $P \leq 0.05$.

7 days experiment. The urine contained more amounts of ametryn than t he feces. By the first day after dosing, the urine contained 0.47% whereas the feces accounted for 0.11% of the applied dose. At the end of the 7 days experimental period, the urine accounted for 3.65% and the feces for 0.55% of the total dose. Thus less than 5% of the dose was eliminated as ametryn during the 7days period. This can be attributed to rapid metabolism of the compound to polar metabolites which are efficiently eliminated. Evidence for this is provided by the work of Oliveret al, 1969, who found that radiolabelled ametryn is highly excreted in the urine than feces. Also they concluded that an ingested dose of ametryn would be rapidly eliminated.

Pharmacokinetics of ametryn

The pharmacokinetic profile of ametryn in the rat is summarized in table 4. Fifteen minutes after dosing, ametryn concentrations reached a peak of 0.60 and 0.15 ug/g for brain and plasma, respectively, indicating very rapid absorption.
Ametryn level in the brain and plasma (Ft) declined monoexponentialy according to the equation 1 and 2

$$Ft\ brain = 0.74\ e^{-0.19t} \quad \ldots\ldots\ldots\ldots (1)$$

$$Ft\ plasma = 0.16\ e^{-0.17t} \quad \ldots\ldots\ldots\ldots (2)$$

The apparent elimination phase of brain and plasma had rate constants (Ke) of 0.19 and 0.17 day-1, respectively. The biological half-life values for brain and plasma were 3.58 and 4.00 days, respectively. These low values are refered to as the fast disposition

TABLE 4

Pharmacokinetic parameters of ametryn in female rats following a single oral dose of 10 mg/kg.

Kinetic parameters	Value	
	Brain	Plasma
Peak conc. ug/g(ml)	0.60	0.15
A ug/g(ml)	0.74	0.16
t1/2, day	3.58	4.00
Ke , day-1	0.19	0.17
AUC mg.hr/l(kg)	1680	240

A=The central compartment of a one-compartment system.
t1/2 = The half-life value.
Ke = The elimination rate constant.
AUC = The area under the curve.

phase and reflect the deposition and distribution of the observed compound from the central compartment into the body tissues. The area under the curve (AUC) values were 1680 and 240 mg. hr/l(kg) for brain and plasma, respectively. As a result of the above data, it was concluded that ametryne would not be stored or accumulated in the body tissues of the rat.

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دراسة حركية واخراج مبيد الاميترين فى الفأر الكبير

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الملخص العربى

تمت هذه الدراسة بغرض تتبع حركية مبيد الأميترين فى الفأر وكذلك معدل اخراجه من الجسم ، وقد تم معاملة انثى الفئران بمبيد الأميترين عن طريق الأنبوبة المعدية بجرعة واحدة قدرها ١٠ مجم / كجم مذابة فى زيت الذرة ، قسمت الحيوانات الى مجموعتين ، تم تخدير المجموعة الأولى ثم سحبت منها عينات الدم عن طريق عمل ثقب فى القلب ثم تم استخراج المخ وذلك بعد ٩ فترات من المعاملة (٠ر٢٥ ، ١ ، ٦ ، ١٢ ، ٢٤ ، ٤٨ ، ٧٢ ، ٩٦ ، ساعة وكذلك ٧ يوم) . أما المجموعة الثانية فقد تم تجميع عينات البول والبراز منها يوميا وذلك لمدة ٧ أيام .

تم تقدير كمية الأميترين المتواجدة فى كل من البلازما والمخ والبول والبراز وذلك باستخدام جهاز التحليل الكروماتوجرافى الغازى ، وقد أظهرت النتائج مايلى :ـ

المبيد سريع الامتصاص فى الجسم حيث تم اكتشافه فى كل من البلازما والمخ بعد ١٥ دقيقة من المعاملة وكان تركيز المبيد فى المخ أعلى منه فى البلازما عند جميع الأزمنة المختبرة ، هذا وقد وصل تركيز المبيد الى ذروته بعد ١٢ ساعة من المعاملة (٠ر١٥ ميكروجرام/مل) والذى يقدر بحوالى ٠ر٠٣٪ من الجرعة المعاملة بها بينما وصل المبيد فى المخ الى ذروته بعد ٢٤ ساعة من المعاملة (٠ر٦٠ ميكروجرام/جم) والذى يقدر بحوالى ١٠٪ من الجرعة المعاملة بها ثم بعد ذلك بدأ التركيز فى التناقص بمرور الوقت حتى وصل الى ٠ر٠١ ، ٠ر١٩ ميكروجراما فى كل من البلازما والمخ على التوالى وذلك بعد ٧ يوم من المعاملة .

أما عن معدل اخراج المبيد فى كل من البول والبراز فقد وجد أنه يتزايد ببطئ بعد اليومين الأول والثانى من المعاملة ثم بدأ يتناقص ببطئ حتى اليوم السابع بعد المعاملة ، وكانت الكمية التى تم اخراجها خلال البول والبراز معا حوالى ٤ر٢ من الجرعة المعاملة بها الفئران وذلك بعد انتهاء اليوم السابع من المعاملة ٠

كان اختفاء الأميترين من كل من البلازما والمخ بمعدل احادى الاس وكان نصف عمر الحياة النهائى حوالى ٣ر٥٨ ، ٤ يوما لكل من المخ والبلازما على التوالى ٠ وكانت القيمة الموجودة تحت منحنى التركيز تمثل ١٦٨٠ ملليجرام ٠ ساعة/كجم ، ٢٤٠ ميلليجرام ٠ ساعة/لتر فى كل من المخ والبلازما على التوالى مما يوضح أن المبيد المستخدم لايتم تخزينه فى الدم اذا ما قورن بالمخ ٠