Bull. Environ. Contam. Toxicol. (1989) 43:66–73

Environmental Contamination and Toxicology

Effects of Paraquat on Parent Generation Female and F_1 Suckling Mice Using Different Treatment Regimes

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The herbicide paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride) is used extensively in commercial agriculture throughout the world. Field paraquat residues have been reported to be as high as 1,000 parts per million (ppm) (Calderbank et al. 1968). Paraquat can be highly toxic to man (Bullivant 1966) and animals (Clark et al. 1966). Death from paraquat poisoning is usually due to respiratory failure brought on by pulmonary fibrosis (Clark et al. 1966). One mechanism of action of paraquat toxicity that has been proposed is that of an interaction of paraquat with oxygen which subsequently induces lipid peroxidation damage of cellular membranes (Bus et al. 1974).

Paraquat has been found to affect reproductive success in bobwhite quail (Bauer, 1985), rats (Daniel and Schreiweis 1977), mice (Bus and Gibson 1975; Dial and Dial 1987), and frogs (Dial and Bauer 1984). Dial and Dial (1987) found significant effects on parent (P) generation female, suckling and weanling mortality and lung tissue following administration of 125 mg/kg paraquat, in food, to pregnant mice then to the young to 49 days post partum.

The current study was designed to ascertain paraquat effects on P females using different treatment regimes and to clarify the cause of suckling mortality. A series of cross-fostering experiments was performed to evaluate suckling mortality resulting from paraquat transfer via the placenta and via milk.

MATERIALS AND METHODS

The following procedures were used for parts 1 and 2 given below. Virgin ICR mice, an outbred strain (Harlan Sprague Dawley, Inc., Indianapolis, IN), were mated at 65 days of age. Several hundred females were randomly divided into groups of 5--which were placed together with one male. Females were checked for vaginal plugs 6 hrs. after pairing and the following 24 hr. period was designated as day 0 of pregnancy when vaginal plugs were found. Nominal concentrations of 0 or 125 mg paraquat cation/kg feed (commerical

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paraquat formulation was used) was administered to mice when appropriate. Diet preparation involved using finely ground Purina lab chow into which 0 or 125 mg/kg paraquat was mixed. Water was added until the feed-paraquat mixture was moist, then hand-squeezed pellets were formed and allowed to dry 2 days at 22.2 ± C. This procedure resulted in an even distribution of paraquat throughout the food. Food was prepared weekly. Food and water were available ad libitum throughout the study. The mice were housed singly, following the finding of vaginal plugs, in clear plastic shoe box cages. Room temperature was 22.2 ± C, and the light cycle of 12L:12D was maintained throughout the study.

The date of parturition and number of young born were recorded for each female. Females were weighed at pairing, parturition, and day 19 post partum. Cages were checked daily for evaluation of the females' health, and adult and F_1 mortality. Both parts of the study were terminated on day 19 post partum.

Lungs were excised from sucklings and adults in groups in which mortality occurred. The tissues were fixed in 10% formalin, paraffin embedded, sectioned at 7 microns, stained with hematoxylin and eosin, and examined by light microscopy.

Part 1--Fifteen females were randomly assigned to each of the following groups:

a-1) control
b-1) paraquat exposure from day 13 of gestation to day of parturition
c-1) foster mothers
d-1) paraquat exposure from day of parturition to day 19 post partum

In a-1: the offspring were raised by their own untreated mothers; no paraquat transfer to F_1
In b-1: the offspring of treated mice were raised by untreated foster mothers; placental paraquat transfer to F_1
In c-1: the offspring of untreated mice were raised by mice exposed to paraquat from day 13 of gestation to day of parturition
In d-1: the offspring were raised by their own mothers with paraquat exposure from parturition to day 19 post partum; milk paraquat transfer to F_1

In groups b-1 and c-1 the females gave birth on the same day; thus litters were of similar age when the cross-fostering took place.

Part 2--Fifteen females were randomly assigned to each of the following groups:

a-2) control
b-2) paraquat exposure from pairing to day of parturition
c-2) foster mothers
d-2) paraquat exposure from pairing to day 19 post partum

In a-2: the offspring were raised by their own untreated mothers; no paraquat transfer to F_1

In b-2: the offspring of treated mice were raised by untreated foster mothers; placental paraquat transfer to F_1

In c-2: the offspring of untreated mice were raised by mice exposed to paraquat from pairing to day of parturition

In d-2: the offspring were raised by their own mothers with paraquat exposure from parturition to day 19 post partum; placental and milk paraquat transfer to F_1

In groups b-2 and c-2 the females gave birth on the same day; thus litters were of similar age when the cross-fostering took place.

The number born per litter, F_1 mortality data, and P female body weights were compared using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test. The Chi-square test was used to compare P female mortality. The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

A summary of data collected for part 1 is given in Table 1. No significant differences were found in the number of young produced per litter in the various groups. F_1 mortality in the group in which paraquat was administered from the day of parturition to day 19 post partum was significantly higher than controls. All F_1 deaths, except 2, in this group resulted from the P females' death. A significant difference in day 9 to day 19 F_1 mortality was observed in the above group.

No P female mortalities occured in the control or foster mother groups nor in the group in which paraquat was administered beginning on day 13 of gestation. However, a significant P female mortality of 40% occurred an average of 14 days post partum in the group in which paraquat was administered from the day of parturition to day 19 post partum. There were no differences in the various groups in P female weights compared to controls.

A summary of data collected for part 2 is given in Table 2. No significant differences were found in the number of young produced per litter in the various groups. F_1 mortality in the group in which paraquat was administered from pairing to day 19 post partum was significantly higher than controls. A significant difference in day 0 to day 8 F_1 mortality was found in this group. All F_1 deaths in the foster mothers' litter, except 1, resulted from the deaths of the paraquat treated cross-fostered P females, which died an average of 4.7 days post partum.

Three types of F_1 mortality were observed in the group in which paraquat exposure began at pairing and continued to day 19 post partum. The types occured as follows: Type I--F_1 mortality when P female appeared healthy and was observed nursing the offspring; Type II--F_1 mortality resulting from P females' poor maternal care,

Table 1. Summary of reproductive performance and mortality of control mice[a], mice fed 125 mg/kg paraquat from day 13 of gestation to parturition[b], foster mothers[c], and mice fed 125 mg/kg paraquat from parturition to day 19 post natal[d]

	Treatment Group			
	C	G	FM	P
No. females on treatment	15	15	15	15
Mean no. in litter*	10.7 ± 0.6[e]	11.5 ± 0.6	10.9 ± 0.9	10.7 ± 0.8
% F_1 mortality by 19 days*	1.9 ± 1.1	4.0 ± 1.5	6.7 ± 2.0	37.3 ± 4.8[f]
% F_1 mortality day 0-day 8*	0.6 ± 0.6	3.5 ± 1.4	6.1 ± 1.9	1.2 ± 0.9
% F_1 mortality day 9-day 19*	1.3 ± 0.9	0.6 ± 0.6	0.7 ± 0.7	36.5 ± 4.8[f]
% P female mortality**	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	40.0 ± 12.6[f]

*ANOVA, Duncan's multiple range test
**Chi-squared
[a]Column labeled C
[b]Column labeled G
[c]Column labeled FM
[d]Column labeled P
[e]Arithmetic mean +/- SE
[f]Different from controls, $P < 0.05$

in this situation the P females exhibited signs of paraquat poisoning; Type III--F_1 mortality resulting from the P females' death. Mortality types I, II, and III were observed in 4, 2, and 5 litters, respectively. In one litter both Types I and II were observed. The mean days of F_1 mortality for mortality Types I, II, and III were 2.4, 8.1 and 7.4, respectively. The mean day of Types I and II was 5.5. The mean day of Type I mortality was 2.3 in the group in which paraquat exposure began at pairing and terminated at parturition; the remaining F_1 deaths in this group were type III deaths resulting from the death of one foster mother.

A significant number of P females died in the group in which paraquat exposure began at pairing and continued to day 19 post partum. No control P females died, however, one foster mother died 12 days post partum. The mean day of death varied between P females exposed to paraquat. Mean mortality days of 4.7 and 7.2 were found for P females with paraquat exposure from pairing to parturition and P females with paraquat exposure from pairing to day 19 post partum, respectively. The only significant difference in P female weights compared to controls was found in the group in which paraquat was administered from pairing to 19 days post partum. In this group, females weighed less at 19 days post partum than controls.

Table 2. Summary of reproductive performance and mortality of control mice[a], mice fed 125 mg/kg paraquat from pairing to parturition[b], foster mothers[c], and mice fed 125 mg/kg paraquat from pairing to day 19 post partum[d]

	Treatment Group			
	C	A	FM	Z
No. females on treatment	15	15	15	15
Mean no. in litter*	10.4 ± 0.4[e]	11.7 ± 0.7	10.8 ± 0.8	11.9 ± 0.6
% F_1 mortality by 19 days*	1.3 ± 0.9	16.5 ± 3.1	24.1 ± 3.9	58.1 ± 5.7[f]
% F_1 mortality day 0-day 8*	0.6 ± 0.6	9.1 ± 2.3	16.1 ± 3.2	36.9 ± 4.5[f]
% F_1 mortality day 9-day 19*	0.6 ± 0.6	8.1 ± 2.3	9.6 ± 2.7	33.6 ± 5.5
% P female mortality**	0.0 ± 0.0	20.0 ± 10.3	6.7 ± 6.5	33.3 ± 12.2[f]

*ANOVA, Duncan's multiple range test
**Chi-squared
[a]Column labeled C
[b]Column labeled A
[c]Column labeled FM
[d]Column labeled Z
[e]Arithmetic mean +/- SE
[f]Different from controls, $P < 0.05$

Light microscopic examinations of lung sections from dead P females exposed to 125 mg/kg paraquat revealed extensive fibrosis. Control P female lungs did not show any significant pathological changes. Histological examination of lungs from 2 day and older sucklings which died in the group in which paraquat was administered from pairing to parturition (placental paraquat transfer to F_1) and the group in which paraquat was administered from pairing to day 19 post partum (placental and milk paraquat transfer to F_1) revealed fibrosis and a corresponding reduction in the number of alveoli compared to control sucklings of the same age in which the lungs were unremarkable histologically. Histological examination of lungs from sucklings which died in the group in which paraquat was administered from parturition to day 19 post partum (milk paraquat transfer to F_1) revealed fibrosis and a reduction of alveoli in a few lungs. Nearly all suckling deaths in this group resulted from death of the P female. There were more alveoli in the affected lungs in this group than in lungs of sucklings receiving paraquat via the placenta (Fig. 1).

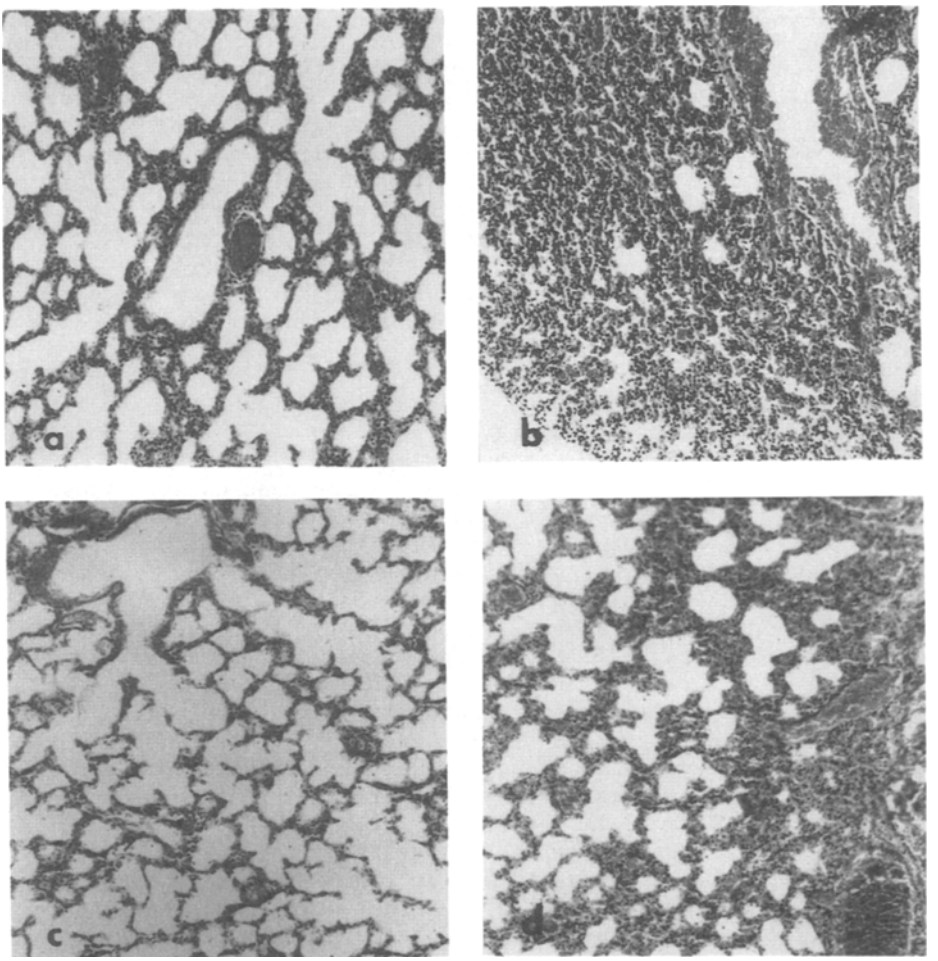

Figure 1. a) Lung of three day old control; b) Lung of 3 day old suckling exposed to paraquat via the placenta; c) Lung of 12 day old control; d) Lung of 12 day old suckling exposed to paraquat via milk. All X125.

The effects on mortality are the results of administration of paraquat in food at levels considerably below those reported after paraquat treatment in the field at the highest recommended application level.

In the present study, feeding paraquat to mice did not appear to be abortifacient or to produce an increase in dead and resorbed fetuses as the number of young produced by treated animals did not differ from controls. This is consistent with the findings of Dial and Dial (1987) who reported that paraquat was not embryotoxic or fetotoxic in mice. Mice did not exhibit any gross malformations in this study nor in previous studies by Dial and Dial (1987) and Bus and Gibson (1975).

In the first part of the present study significant F_1 mortality was found by 19 days post partum in the group in which the parent mice were placed on paraquat from the day of parturition to day 19 post partum (milk paraquat transfer to F_1). All, except two, of the F_1 deaths resulted from the death of the P female and took place after 9 days post partum. Only a few of the suckling lungs revealed fibrosis and more alveoli were observed than observed in lungs of sucklings receiving paraquat via the placenta. The lung fibrosis may not have been extensive enough to cause F_1 mortality in the absence of maternal mortality. No F_1 mortalities could be attributed solely to milk transfer of paraquat. Forty percent of the females placed on paraquat on the day of parturition died by 19 days post partum.

In the second part of the present study significant F_1 mortality was found by 19 days post partum in the group in which the parent mice were administered paraquat from pairing to day 19 post partum. Three types of F_1 mortality were found. The mean day of Types I and II was 5.5. This agrees well with data reported by Dial and Dial (1987) in which the mean day of F_1 suckling mortality was 5.9 (the latter figure disregarded suckling mortality resulting from the P females' death). Nearly all F_1 Type I mortality sucklings died from paraquat lung fibrosis. The mean day of Type I mortality was very similar being 2.4 and 2.3 for the group in which paraquat exposure began at pairing and continued to day 19 post partum and the group in which paraquat exposure began at pairing and terminated at parturition, respectively. Under the latter treatment regime the offspring were exposed to paraquat prenatally but not postnatally. This indicates that paraquat transfer via the placenta and not milk induces Type I F_1 mortality resulting from lung fibrosis. The present data largely supports the suggestion by Bus and Gibson (1975) that suckling mortality may be linked to the initiation of breathing and exposure of lungs to higher oxygen tensions and that paraquat from prenatal placental transfer and not from the mother's milk is the likely route of transfer. Bus et al. (1975) found that paraquat administered to pregnant rats during the last two days of gestation was detectable in newborns for up to 7 days postnatally. It has been shown that increased oxygen tensions increased paraquat toxicity (Fisher et al. 1973) and oxygen has been linked to the mechanism of action of paraquat toxicity via a proposed interaction of paraquat with oxygen which subsequently induces lipid peroxidation damage of cellular membranes (Bus et al. 1974).

In the current study, paraquat induced significant F_1 and P female mortality when administered under different treatment regimes. Paraquat was not embryo/fetotoxic, however, very young sucklings which were exposed to paraquat only prenatally, via the placenta, died from paraquat induced lung fibrosis. It is believed that the current study further substantiates the importance of the role of oxygen in inducing paraquat toxicity.

Acknowledgment. This research was supported in part by grant 2-29214 from the University Research Committee, Indiana State University.

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Received September 18, 1988; accepted November 5, 1988.