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TOXICITY OF HERBICIDES 2,4-D AND MCPA FOR RATS AND RABBITS

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Abstract

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A subacute toxicity test using 2,4-dichlorophenoxyacetic acid (2,4-D, 10 mg·kg^{-1} and 100 mg·kg^{-1}) and 4-chloro-2-methylphenoxyacetic acid (MCPA, 15 mg·kg^{-1} and 150 mg·kg^{-1}) was carried out on Wistar rats (n = 60) and New Zealand White rabbits (n = 30) of both sexes.

The lower doses of 2,4-D and MCPA used in our experiments were chosen based on the estimate that such doses would be present in feed of plant origin treated with the concentrations of both herbicides recommended by the manufacturer; the higher ones were sublethal doses.

Herbicides 2,4-D and MCPA in our trial did affect neither body weight in adult rats and rabbits and their offspring nor weights of inner organs or the red and white blood cell count. The mucous membrane of the stomach and small intestines in rats treated with high doses of both herbicides was slightly inflamed and swollen. In rabbits, some of the semen characteristics changed during the trial; both herbicides acted in different directions. At the end of the trial the concentration of serum testosterone was significantly higher ($p < 0.05$) in rabbits treated with MCPA than in controls. The number of surviving young per litter at weaning in the MCPA group of rabbits was lower than in controls. No evident toxic effects on the viability and the ossification processes were observed in the young of treated rabbits.

Aryloxyacetic acid-derivative herbicides, plants, recommended dose, subacute toxicity, reproduction toxicity

Substances used as herbicides should exert highly selective effects on plants, and have low toxicity for micro-organisms, fish, insects, domestic and wild animals, and humans. In acute poisoning of rats, poultry, cattle, dogs, and humans with aryloxyacetic acid derivative herbicides, cases of metabolic acidosis, respiratory alkalosis, neuropathy, myopathy (Beasley et al. 1991; Paggiaro et al. 1974; Park et al. 1977; Prescott et al. 1979), bradycardia, changes in electrocardiogram (Prokofyeva 1988), a drop in acetylcholinesterase activity and necrosis of the muscles (Bernard et al. 1985), changes in blood constituent values (Prokofyeva 1988), necrosis of the liver, hyperplasia of Bowman's capsule, and adenomas (Loktionov 1986) were repeatedly diagnosed. A dose of 200 mg·kg^{-1} of body weight of the 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide has been cited as exceptionally toxic for cows, causing apathy, anaemia of the mucous membranes, along with a drop in body temperature (Loktionov 1986).

In chronic poisoning of poultry and pregnant sows motor disorders, albuminuria, degeneration of liver and kidney appeared, and increased mortality among newborn piglets or reduced egg production in poultry were found (Loktionov 1986).

Administration of aryloxyacetic acid-derivative herbicides during organogeny led to disorders in foetuses such as dropping eyelids, hydrocephalus, split hard palate, anomalies of the heart, kidneys, and skeleton (Abbott et al. 1989; Birnbaum et al. 1989; Courtney et al. 1970; Courtney and Moore 1971; Emerson et al. 1970; Khera et al. 1971; Loktionov and Tsyrempilov 1987; Vos et al. 1974).

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In the synthesis of aryloxyacetic acid-derivative herbicides, tetrachlorodibenzo-p-dioxin (TCDD), having a teratogenic effect, is formed as a by-product (Abbott and Birnbaum 1989; Abbott and Birnbaum 1990; Bernard et al. 1985). The maximum permitted amount of TCDD in herbicides is 0.1 mg·kg^{-1} of herbicide (Janjič 1985). In rats treated with TCDD the serum concentration of testosterone was found to be 10 -75% lower than in non-treated animals (Kleeman et al. 1990), whereas secretion of the LH hormone was not changed (Bookstaff et al. 1990a; Bookstaff et al. 1990b). Also decreased concentrations of cortisone and of thyroid hormones T3 and T4 were observed (Hermansky et al. 1988; Roth et al. 1988).

MCPA and 2,4-D were found to be slightly mutagenic at concentrations between 250 and 750 µg/plate in the strain *Salmonella typhimurium* TA 97 (Kappas 1988).

In the present study we determined whether sublethal doses of aryloxyacetic acid derivative herbicides 2,4-D and MCPA, substances categorised as plant hormone herbicides - were able to produce toxic effects in either adult rats and rabbits or in their offspring. We were particularly interested in the possible effect of the herbicides on processes linked to reproduction in females and males as well as on foetuses, and the development of the young until weaning. For this purpose we chose the doses of 2,4-D and MCPA which could be contained in the feed of plant origin treated with the concentrations of both herbicides as recommended by the manufacturer.

Materials and Methods

We carried out two sets of experiments on 60 Wistar rats and 30 New Zealand rabbits, using the herbicides 2,4-D and MCPA.

Experiments on rats

Sixty Wistar rats (30 females and 30 males aged two months, body weight 200 - 230 g) were divided into four test groups and one control group, each comprising five females and five males in the control group, ten males and ten females were included. The groups of animals received
2,4-D, 100 mg·kg^{-1} b. w.,
2,4-D, 10 mg·kg^{-1} b. w.,
MCPA, 150 mg·kg^{-1} b. w.,
MCPA, 15 mg·kg^{-1} b. w.,
tap water (control group).

Experiments lasted 90 days for male rats. In females, experiments with low doses of 2,4-D and MCPA lasted 45 days (30 days before and during mating, and 10 days after the last mating day), and in those with high doses 35 days (30 days before and during mating). Animals were administered either aqueous solution of the herbicide or clean tap water (control group) in equal volumes of 1 ml·kg^{-1} of body weight orally onto the root of the tongue with a stomach tube, each second day.

Experiments were carried out on two-month-old sexually mature females that were kept with males of the same age (one male to five females of the same group). After five days together the males were returned to the cages holding males in a single group. On day 15 of pregnancy, the females were moved to cages with barriers for the individual parturition. The administration of herbicides began one week after transfer of the animals.

The adult rats were sacrificed one to three days following administration of the last herbicide dose, and all rat offspring on day 21 after birth. All rats were anaesthetised by ether and bled to death.

Experiments on rabbits

In experiments on rabbits only low herbicide doses were used.

Thirty New Zealand White rabbits (15 females aged five months and 15 males aged six months, body weight 2940 - 4070 g) were divided into three groups with five females and five males in each. These groups of animals received
2,4-D 10 mg·kg^{-1} b. w.,
MCPA 15 mg·kg^{-1} b. w.,
tap water (control group).

The females were included into the experiment after first parturition. They were individually bred with the males of the same group, each female bred twice with the same male.

The experiment using males lasted 90 days, the experiment using females lasted 48 days (30 days before and 18 days after mating). Animals received aqueous solution of the herbicide or tap water, 1 ml·kg^{-1} b. w., on the root of the tongue with a stomach tube, every second day.

The adult rabbits were sacrificed one to three days after the administrations of the last dose, and the young on the 28th day after their birth. All rabbits were anaesthetised by ether and bled to death.

In the study the following values were recorded:
- once per week body weights of adult rats and rabbits and their young;
- erythrocyte and leukocyte counts, haemoglobin, haematocrit in rats and rabbits at the end of the trial;
- number and vitality of the offspring born to treated rats and rabbits;
- serum testosterone concentration in male rabbits;
- macroscopic and microscopic properties of the rabbit semen;
- pathoanatomical examination of organs of rats and rabbits;
- absolute and relative weights of organs of sacrificed adult rats and rabbits;
- pathohistological examination of the radii of rabbit young.

During the experiment, the adult rats were weighed every 7th day, and their young on days 7, 14, and 21 after birth. Body weights of adult rabbits were also recorded every 7th day, and of their young on day 5, 12, 19, and 28 after birth.

Herbicides 2,4-D and MCPA were administered to the animals in the form of aqueous solution of the preparations Deherban A and Deherban M, respectively (Chromos, Zagreb, Croatia). The doses mentioned in the text refer to doses of pure 2,4-D or MCPA and were calculated from the composition of active ingredients in Deherban A and Deherban M preparations as declared by the manufacturer: Deherban A contains 464 g 2,4-D (purity 98%), and Deherban M 450 g MCPA (purity 98%) in one litre of solution.

Blood samples in rats were taken from the jugular vein when animals were sacrificed, and in rabbits from the auricular vein before they were sacrificed at the end of the trial.

In the blood samples, the erythrocyte and leukocyte counts, haemoglobin, and haematocrit were measured by the Coulter cell counter. Differential leukocyte blood count (segmented neutrophils, band neutrophils, eosinophils, basophils, lymphocytes, and monocytes) was determined on blood smears stained by Pappenheim (Slanina et al. 1993). The obtained values were compared with those of controls.

Concentration of testosterone in the serum of male rabbits was determined by RIA method (using the commercial kits, Diagnostic Products Corporation, Los Angeles). For this purpose blood samples were taken from the auricular vein of male rabbits one week after moving the animals, and at the end of the trial.

Semen samples were taken from male rabbits using an artificial vagina before the beginning of the experiment and eight times during the experiment. On the basis of two ejaculation samples taken a week apart, the beginning quality of the semen and the libido of individual male rabbits were determined. Semen smears were stained according to Hancock (1952). Prepared slides were evaluated under the microscope using the 500-fold immersion magnification.

In individual samples of semen of rabbits in both test groups and controls the following macroscopic and microscopic characteristics were evaluated: volume, viability, mucus, colour, density, total number of spermatozoa, changes of their head, neck, middle part and tail, and damage to acrosome. The relationships between these characteristics were also evaluated.

For the histological preparation, radii of the rabbit young were stained with hematoxylin and eosin, and examined by a light microscope using 400-fold magnification.

The distal and proximal lines of epiphyseal growth of the right leg radii of 15 randomly chosen young from each group were examined. The epiphyseal growth lines were analysed from the epiphyseal to the diaphyseal edge.

Statistical analysis

The mean value (x) and either standard deviation (SD) or standard error of the mean (SEM) were calculated for each variable measured. Significance of differences between means was calculated using Scheffe's-test. A one-way ANOVA for repeated measurements was used to analyse the effects of given drugs. Results with $p < 0.05$ or lower were considered as statistically significant.

The experiments were approved by the Ministry of Agriculture, Forestry and Food, and by the Veterinary Administration of the Republic of Slovenia.

Results

Body and organ weights of sacrificed animals

There were no significant differences between the test groups and control group of rats in body weight gain during the experiment or in weight of individual organs upon sacrifice. There were also no significant differences in body weights of the offspring born to the control group of rats and those born to the test groups of rats as measured on days 7, 14, and 21 after birth.

In body weights of adult rabbits and their offspring we found substantial individual

differences, as well as great fluctuations during the experiment. However, there were no significant differences in body weights between the control and the test group of adult rabbits and their offspring.

The weights of individual organs in the adult rabbits and their young in the control group did not significantly differ from those in either of the test groups.

Evaluation of offspring born to treated rats and rabbits

In the control group and in both groups treated with 2,4-D 70 % of female rats placed together with a male had young. In groups of rats treated with MCPA, 60 %, respectively. The average numbers of young per litter in the control group, in groups treated with 2,4-D, and in groups treated with MCPA were 9.7, 7.1, and 11, respectively. The survival rates in all groups were 100 %.

All female rabbits placed together with a male had young. In the control group of rabbits, on average, 7.6 young were born per litter, and 94.7% of the young survived until weaning. Female rabbits that received 2,4-D at the doses of 10 mg·kg b. w. gave birth on average to 6.8 young, and 88.2% of these young survived until weaning. In female rabbits treated with MCPA at the dose of 15 mg·kg b. w., on average 9.0 young were born per litter, with 66.7% surviving until weaning (Fig.1).

Table 1
Hematological profile of the rats treated with 2,4-dichlorophenoxyacetic acid (2,4-D) or 4-chloro-2-methylphenoxyacetic acid (MCPA) (x ± SD, n = 5, Er - erytrocytes, LC - leukocytes, Hb - haemoglobin, Hc - haematocrit, Ns - segmented neutrophils, Nb - band neutrophils, Eo - eosinophils, Ba - basophils, Ly - lymphocytes, Mo - monocytes)

Group	Er n/L×10^{12}	Lc n/L×10^{9}	Hb nmol/L	Hc l/l	Ns %	Nb %	Eo %	Ba %	Ly %	Mo %
Control females	7.53 ±0.88	7.60 ±3.42	15.24 ±1.21	0.484 ±0.06	8 - 25	0 - 1	0 - 6	0 - 4	63 - 86	0 - 4
Control males	8.19 ±0.92	11.76 ±8.70	15.66 ±0.98	0.490 ±0.06	15 - 59	0 - 4	0 - 2	0 - 2	36 - 83	0 - 5
2,4D 100 mg·kg^{-1} females	6.24 ±1.87	9.94 ±3.22	13.30 ±2.59	0.394 ±0.08	8 - 30	0 - 4	0 - 3	1 - 3	66 - 89	0 - 1
2,4D 100 mg·kg^{-1} males	8.52 ±0.42	9.54 ±2.25	16.30 ±0.84	0.486 ±0.04	18 - 26	0 - 2	0 - 4	0 - 2	72 - 79	0 - 3
2,4D 100 mg·kg^{-1} females	7.24 ±0.23	5.12 ±1.19	14.70 ±0.68	0.426 ±0.02	11 - 20	0 - 3	0 - 3	0 - 5	70 - 84	0 - 3
2,4D 100 mg·kg^{-1} males	8.78 ±1.87	8.44 ±3.22	16.06 ±2.59	0.484 ±0.08	15 - 29	0 - 1	0 - 3	0 - 1	64 - 83	0 - 4
MCPA 100 mg·kg^{-1} females	6.98 ±0.92	5.44 ±0.52	14.26 ±0.87	0.432 ±0.06	10 - 22	0 - 4	0 - 1	0 - 3	75 - 83	0 - 3
MCPA 100 mg·kg^{-1} males	8.66 ±0.45	8.02 ±1.75	16.22 ±1.22	0.516 ±0.06	15 - 26	0 - 3	69 - 85	0 - 1	69 - 85	0 - 3
MCPA 100 mg·kg^{-1} females	7.40 ±0.16	5.82 ±0.74	15.42 ±0.75	0.447 ±0.03	10 - 21	0 - 1	0 - 2	0 - 2	76 - 86	0 - 3
MCPA 100 mg·kg^{-1} males	8.10 ±0.00	9.95 ±4.45	15.60 ±0.14	0.465 ±0.07	9 - 12	0 - 1	1 - 4	1 - 2	83 - 86	0 - 1

Table 2
Hematological profile of the adult and young rabbits treated with 2,4-Dichlorophenoxyacetic acid (2,4-D) or 4-chloro-2-methylphenoxyacetic acid (MCPA) (x ± SD, n = 5, n*=15, Er - erytrocytes, LC - leukocytes, Hb - haemoglobin, Hc - haematocrit, Ns - segmented neutrophils, Nb - band neutrophils, Eo - eosinophils, Ba - basophils, Ly - lymphocytes, Mo - monocytes)

Group	Er n/L×10^{12}	Lc n/L×10^{9}	Hb nmol/L	Hc l/l	Ns %	Nb %	Eo %	Ba %	Ly %	Mo %
Control females	5.20 ±0.23	7.40 ±4.82	11.02 ±1.72	0.354 ±0.03	4 - 35	0	0 - 4	0	41 - 96	0 - 3
Control males	5.80 ±0.35	8.88 ±3.27	13.56 ±0.79	0.398 ±0.02	6 - 14	6 - 14	0 - 6	0 - 1	81 - 88	0 - 1
Control young*	4.85 ±1.87	11.08 ±3.22	11.93 ±2.59	0.362 ±0.08	5 - 41	0 - 4	0 - 13	0 - 1	51 - 90	0 - 6
2,4D 10 mg·kg^{-1} females	4.94 ±0.25	8.14 ±4.67	11.14 ±0.51	0.340 ±0.01	0 - 23	0	1 - 9	0 - 1	74 - 93	0 - 2
2,4D 10 mg·kg^{-1} males	5.64 ±0.26	7.20 ±1.58	13.80 ±1.10	0.390 ±0.01	0 - 19	0	0 - 4	0 - 2	79 - 93	0 - 4
2,4D young*	4.71 ±0.44	15.40 ±8.51	12.29 ±1.59	0.346 ±0.05	2 - 23	0 - 2	1 - 11	0 - 2	69 - 90	0 - 6
MCPA 15 mg·kg^{-1} females	4.98 ±0.92	7.70 ±0.52	11.02 ±0.87	0.362 ±0.06	5 - 43	0 - 1	0 - 8	0 - 1	48 - 94	0 - 1
MCPA 15 mg·kg^{-1} males	8.66 ±0.40	8.02 ±2.11	16.22 ±0.72	0.516 ±0.03	15 - 26	0 - 3	69 - 85	0 - 1	69 - 85	0 - 3
MCPA young*	4.40 ±0.43	15.79 ±6.58	10.93 ±0.79	0.362 ±0.04	4 - 39	0 - 1	0 - 5	0 - 2	53 - 96	0 - 3

Analysis of blood samples from sacrificed animals

The administration of herbicides 2,4-D or MCPA in our experiment did not significantly affect the red or white blood cell counts neither in rats nor in rabbits, as compared to controls. However, there were great differences in leukocyte counts within all groups (Tables 1 and 2).

In our experiments, females of all groups and of both species always had a somewhat lower erythrocyte counts and a lower amount of haemoglobin than males.

Similarly, the haemoglobin and haematocrit values did not differ significantly in any of the treated group of either species from the corresponding values of controls. These indices in the young of treated rabbits were not significantly different from those of control young either.

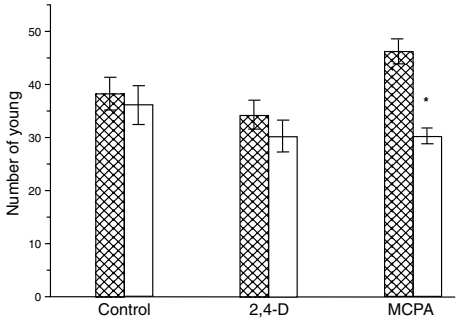

Fig. 1. Average number of rabbit young at birth ⊠ and at weaning □. Parents treated with (10 mg·kg^{-1}) or MCPA (15 mg·kg^{-1}). * $p<0.05$ vs. number at birth.

The concentration of testosterone in the blood serum of adult rabbits

The concentration of testosterone in control group of rabbits and in those treated with 2,4-D was significantly lower ($p < 0.01$) at the end of the experiment than at the beginning. However, the drop in the concentration of testosterone was not significant in rabbits treated with MCPA

(Table 3). At the end of the trial the concentration of serum testosterone was significantly higher ($p < 0.05$) in rabbits treated with MCPA than in control animals (Table 3).

Table 3
Concentratios of testosterone (nmol/L, x ± SD, n = 5) in serum of rabbits treated with 2,4-dichlorophenoxyacetic acid (2,4-D) 10 mg·kg^{-1}, or 4-chloro-methylphenoxyacetic acid (MCPA) 15 mg·kg^{-1} on Day 0 and on Day 90 of experiment (in brackets range of value per group)

Group	Day 0 of experiment	Day 90 of experiment
Control	52.53 ± 28.24 (5.78 - 78.91)	6.69 ± 3.28** (3.73 - 11.98)
2,4-D	54.15 ± 27.80 (14.74 - 76.64)	8.88 ± 8.78** 2.95 - 23.87)
MCPA	34.17 ± 32.13 (7.56 - 80.99)	14.45 ± 7.60* (2.88 - 32.80)

* $p < 0.05$ vs. control
** $p < 0.01$ vs. beginning values

Characteristics of the semen of rabbits

Most of the observed characteristics in the test groups did not differ significantly from those in the control group. During the trial both herbicides significantly changed ($p < 0.05$ to $p < 0.001$) some of the relationships between observed characteristics:

- 2,4-D (10 mg·kg^{-1}) changed the negative correlation between the content of mucus and either intensity of the colour or changes on head or tail to a positive correlation, whereas positive correlations between the content of mucus and changes on neck as well as between intensity of colour and damage to acrosome were changed to the negative at the end of the trial;
- MCPA (15 mg·kg^{-1}) changed the negative correlation between density of the semen fluid and damage to the acrosome to positive. Correlation between changes in the middle part and changes on the tail was turned from positive to the negative;
- MCPA (15 mg·kg^{-1}) turned the negative correlation between damage to the acrosome and changes on the neck at the beginning of the trial to a positive correlation at the end of the trial. The opposite happened in the group treated with 2,4-D (10 mg·kg^{-1}).

Pathoanatomical findings in rats at the end of the trial

Three male rats died (on days 35, 56, and 64 of the experiment) in the group treated with MCPA at the dose of 150 mg·kg^{-1} of b. w., and one female rat died (on day 85 of the experiment) in the group treated with MCPA at the dose of 15 mg·kg^{-1} of b. w.

In all male rats the death was due to aspiration caused by oral herbicide administration with the stomach tube. No pathoanatomical changes were found in the perished female rat.

The macroscopic examination of organs at the pathoanatomical dissection of the rats treated with 2,4-D at doses of 100 mg·kg^{-1}, and with MCPA at doses of 150 mg·kg^{-1} revealed slightly inflamed and swollen mucous membranes of the stomach and small intestines.

The pathoanatomical dissections of the young of rats of all groups that were born during the experiment did not reveal any organ or body deformations.

Pathohistological findings in the radii of the rabbit offspring

The examination of the histological preparations of the radii of the rabbit young of both test groups revealed no differences as compared to controls.

Discussion

Both preparations used in our experiments (Deherban A and Deherban M) contained, with exception of 2,4-D and MCPA, according to the manufacturer's assurance no further toxic

substances. However, the exact composition was not known to us and we carried out the experiments on the assumption that the toxic effects could be ascribed to both active substances only, to 2,4-D and MCPA, respectively.

The lower doses of 2,4-D and MCPA used in our experiments were chosen on the assumption that such doses could be contained in feed of plant origin that had been treated with the concentrations recommended by the manufacturer of these herbicides without consideration of abstinence. The higher doses were sublethal doses.

A shorter time of administration of 2,4-D and MCPA in high doses (100 $mg \cdot kg^{-1}$, and 150 $mg \cdot kg^{-1}$ b. w., respectively) was chosen as our interest was focused on the possible effect of low doses of these herbicides on organogeny in rats.

The overall time of treatment in female rabbits was different from that in female rats because of different time of organogeny in both species (Jung 1958; Kozma et al. 1974).

In our experiment of acute toxicity the administered sublethal doses of herbicides did not have a negative influence on the gain of body weight in the adult test rats and rabbits as it was found by some other investigators (Courtney et al. 1970; Courtney et al. 1971; Yasuda and Maeda 1972), who used similar doses.

The results of evaluation of offspring born to either rats or rabbits (number of offspring, vitality and body weight upon birth up to sacrifice) in our experiments differ from the data by Loktionov and Tsyrempilov (1987). Macroscopic examination of the offspring of both species, either at birth or at sacrifice, did not reveal any of the development disorders reported by other authors (Courtney et al. 1970; Courtney and Moore 1971; Loktionov 1986; Yasuda and Maeda 1972).

Our results are in agreement with those of Emerson et al. (1970) who treated rats with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) at doses ranging from 1 $mg \cdot kg^{-1}$ to 24 $mg \cdot kg^{-1}$ b. w., from the 6th to the 15th day of pregnancy, and of Khera et al. (1971) who treated rats with 2,4,-D and 2,4,5-T at doses of 100 $mg \cdot kg^{-1}$ and 150 $mg \cdot kg^{-1}$ b. w. The administered doses of herbicides in our study did not affect the weight of individual organs in adult rats and rabbits nor the weight of the individual organs of the rabbit young. Atrophy or hypertrophy of organs which was found by Kerkvaliet et al. (1990) and by Loktionov and Tsyrempilov (1987) after treatment of mice and rats with 2,4-D, 2,4,5-T and TCDD was not observed under conditions of our study.

In our experiments the only pathological finding was slightly inflamed and oedematous mucous membrane of the stomach and small intestines in the rats treated with high doses of both herbicides.

The administration of herbicides 2,4-D or MCPA did not affect the blood cell counts neither in rats nor in rabbits. The results were in the range of physiological values (Jung 1958; Kozma et al. 1974).

All the values of the differential white blood count in adult rabbits and their young (Table 2) were within the limits of physiological values for rabbits (Kozma et al. 1974).

In our experiments the females of all groups and of both species always had a somewhat lower erythrocytes counts and a lower amount of haemoglobin than the males (Tables 1 and 2), which can be attributed to physiological differences between the sexes.

Our results differ from those of Košutzký et al. (1990) and Loktionov (1986) whose data indicated that a thirty-day-long administration of 2,4-D at doses of 100 $mg \cdot kg^{-1}$ or MCPA at doses 100-500 $mg \cdot kg^{-1}$ in cows caused a drop in the concentration of haemoglobin, a decrease in the number of erythrocytes and an increase in the number of leukocytes.

In our experimental groups, the average number of young per litter was within the usual limit values for rats (Jung 1958).

The differences in the number of rabbit offspring born, weaned, and lost are not statistically significant except for the group treated with MCPA (Fig. 1). In this group the

number of lost offspring was significantly higher than in controls. All other values were within the normal limits for rabbits (Kozma et al. 1974).

The differences in relationships between individual macroscopic and microscopic characteristics of rabbit semen in control group and both test groups could be ascribed to the action of both herbicides but they could also be the consequence of individual differences between the males.

The blood samples for determining the concentrations of testosterone in serum were taken at the beginning and at the end of the experiment, at the same time of the day. Great differences between single values are typical of such type of analysis because of pulsatile secretion of hormones (Garner and Hafez 1987).

The concentrations of testosterone in serum taken at the end of the experiment from rabbits are comparable to the data of the physiological concentrations of testosterone in rabbits (Ramirez and Bayer 1988). The values of the concentration of testosterone in serum taken from the same animals at the beginning of the experiment, prior to administration of the herbicides 2,4-D and MCPA, were higher than those listed by these authors but still within the physiological range (Garner and Hafez 1987).

The greatest decrease of concentrations of testosterone during the experiment, namely to 13% of the initial value, was found in the rabbit control group; in rabbits treated with 10 $mg \cdot kg^{-1}$ of 2,4-D the concentration fell to 19% of the initial value, whereas the smallest fall was experienced by the rabbits treated with 15 $mg \cdot kg^{-1}$ MCPA, whose concentration of testosterone totalled 42% of the initial concentration (Table 3).

As a decrease in the concentration of testosterone was experienced in all rabbits at the end of our experiment and was particularly marked in the rabbits of the control group, this drop in the testosterone serum concentration cannot be attributed to the effect of both tested herbicides. These results are in accordance with the results reported by Bookstaff et al. (1990ab) and by Kleeman et al. (1990) in rats that were given TCDD.

The pathohistological evaluation of the preparations of the radii of young born to rabbits that received 2,4-D or MCPA did not reveal any changes which would point to disorder of ossification in long bones, such as reported by Sparschu et al. (1970) following administration of TCDD to rats.

In conclusion, findings of our subacute experiment show that oral treatment of rats and rabbits with either 2,4-D at doses of 10 $mg \cdot kg^{-1}$ or 100 $mg \cdot kg^{-1}$, or with MCPA at doses of 15 $mg \cdot kg^{-1}$ or 150 $mg \cdot kg^{-1}$ did change neither body weights in adult rats and rabbits and their offspring nor weights of inner organs nor the red and white blood cell counts. The mucous membranes of the stomach and small intestines in rats treated with high doses of both herbicides was slightly inflamed and swollen. In rabbits, some of the semen characteristics changed during the trial, both herbicides acting in different directions. At the end of the trial, the concentration of serum testosterone was significantly higher in rabbits treated with MCPA group than in control animals. Also the number of surviving young per litter at the weaning was lower in the MCPA group of rabbits than in controls. The vitality and ossification processes of the young born to the tested rabbits were not affected.

Toxicita herbicidů 2,4-D a MCPA pro potkany a králíky

V subaktuním pokusu bylo k testování 2-4-dichlorofenoxyoctové kyseliny (2,4-D, 10 $mg \cdot kg^{-1}$ a 100 $mg \cdot kg^{-1}$) a 4-chloro-2-methylfenoxyoctové kyseliny (MCPA, 15 $mg \cdot kg^{-1}$ a 150 $mg \cdot kg^{-1}$) použito potkanůWistar (n = 60) a novozélandských králíků (n = 30), a to samců i samic. Nižší dávky 2,4-D a MCPA byly použity na základě předpokladu, že se jedná o přibližnou dávkou jakou by zvířata požila při konzumu rostlinné potravy ošetřené těmito látkami dle návodu výrobce. Vyšší dávky byly subletální.

Testované herbicidy neovlivnily živou hmotnost pokusných zvířat ani jejich potomstva,

ani hmotnost jejich orgánů nebo složky červeného a bílého krevního obrazu. Sliznice žaludku a tenkého střeva u potkanů ošetřených vysokými dávkami obou herbicidů byla mirně zanícená a edematózní. U králíků byly změněny některé charakteristiky semene; na konci pokusu byla koncentrace sérového testosteronu významně vyšší u králíků ošetřených MCPA než u kontrol. Počty mláďat ve vrhu, přežívajících do odstavu, byly nižší u králíků ošetřených MCPA. Osifikační procesy u mláďat králíků nevykazovaly známky toxického vlivu herbicidů.

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