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TOLERANCE AND ACCEPTABLE DAILY INTAKE OF ETHYLENE DICHLORIDE IN THE CHICKEN DIET*

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Abstract—Leghorn chickens of both sexes were fed mash fumigated with ethylene dichloride (1,2-dichloroethane) for 2 yr. Growth, performance of layers, fertility, reproduction and biochemical tests served as criteria for establishing the no-effect levels. Ethylene dichloride did not affect the growth, semen characteristics or fertility of the chickens, and the results of serum analyses on treated chickens did not differ from those on controls. Both fumigant levels, 250 and 500 ppm in the mash, caused a moderate but persistent decrease in egg weight from month 4 of the laying period. Egg production was affected only by the higher level of fumigant. A tolerance of 100 ppm and an acceptable daily intake of 5 mg/kg body weight seem to be justified for laying hens. For growing chicks and cocks the tolerance and acceptable daily intake are the same as those previously determined for rats, namely 250 ppm and 25 mg/kg, respectively.

INTRODUCTION

The importance of studying the effect of the prolonged feeding of animals on diets containing fumigant residues has already been emphasized (Alumot, Nachtomi, Mandel, Holstein, Bondi & Herzberg, 1976).

The use of chickens as experimental animals for establishing no-effect levels for man is justified by species differences in the long-term toxicity of fumigants. As mentioned in the introduction to the preceding report (Alumot *et al.* 1976), ethylene dibromide affected such different phenomena as laying activity in hens and spermatogenesis in bulls when administered in both cases at a level of about 1% of the LD_{50} daily for several weeks. On the other hand, reproductive activity in rats, the most widely used species for evaluation of toxicity, was not affected even by larger doses of ethylene dibromide (E. Alumot *et al.* unpublished data). Freerksen & Kazda (1974) proposed recently that the reproductive activity of the laying hen could provide a very sensitive test for the evaluation of drug activity.

The present report deals with observations on chickens fed for 2 yr on diets fumigated with two levels of ethylene dichloride (EDC). EDC is less toxic than ethylene dibromide and was therefore tried in the mash at higher levels than ethylene dibromide.

Carbon tetrachloride could not be tested on chickens as these animals are insensitive to both acute (Hall & Shillinger, 1923) and long-term administration (E. Alumot *et al.* unpublished data) of this compound.

EXPERIMENTAL

The fumigation of the mash, storage conditions and residue determinations, as well as the biochemical tests have been described (Alumot *et al.* 1976). The EDC levels in the mash were 250 ± 30 ppm and 500 ± 40 ppm.

Feeding procedure. Growing birds were fed three meals each day, from 06.30 to 08.30 hr, from 11.00 to 12.00 hr and from 14.00 to 16.00 hr. This regime was chosen after preliminary tests lasting 1 wk, during which the consumption was determined by weighing the mash in the troughs each hour. The controlled feeding resulted in a total consumption no lower than that by continuously fed animals. All the groups consumed the same amount of feed, so in practice paired-feeding was attained by this feeding regime. Laying hens were fed for two 2-hr periods during the day, and a third weighed portion was left overnight to ensure availability of food during egg formation. The accuracy of the paired feeding was thus interfered with during this third feeding. In preliminary experiments, the mash was tested for fumigant loss during the different feeding periods. The losses were about 20% after 1 hr in the feed trough and up to 40% after 2 hr. The average amount of EDC consumed was about 70% of the initial value.

Experimental design and conduct. White Leghorn chicks were divided into three groups, each consisting of ten males and 20 females, and fed control mash or fumigated mash containing either 250 or 500 ppm

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Table 1. *Growth and feed efficiency of Leghorn chicks fed EDC-fumigated mash*

Duration of trial (wk)	Values for chicks fed EDC levels (ppm) of 0		250		500	
	Weight (g)	Feed efficiency*	Weight (g)	Feed efficiency*	Weight (g)	Feed efficiency*
0	73	—	70	—	71	—
1	115	0·47	111	0·52	116	0·54
2	176	0·58	165	0·56	170	0·54
3	245	0·47	233	0·45	240	0·48
5	403	0·37	400	0·38	399	0·37
6	520	0·32	507	0·29	513	0·31
8	739	0·29	718	0·26	706	0·24

*Feed efficiency = (Weight gain/Food consumption)
Values are means for groups consisting of ten males and 20 females.

EDC. Growth and feed consumption were recorded weekly up to 8 wk of age and monthly thereafter.

At the age of 4·5 months, pullets were separated from cockerels and transferred to the laying house. After laying began, the number of pullets was reduced to 16/group by random selection. The pullets were placed in individual laying cages and their performance was recorded daily. Initially, eggs were weighed weekly on a group basis. When the first signs of weight decrease were noted, all the eggs were weighed individually for 1 wk. Thereafter, individual weighing was carried out on three consecutive days, twice each month.

The males were checked for semen production and sperm motility 12 times, twice weekly, when 7–8·5 months old. At the age of 8·5 months, seven cocks from each of the treated groups and five control cocks were placed in individual cages. Semen examination was continued.

The fertility of the cocks and the hens was tested in two trials by means of artificial insemination. In the first trial, half of the hens on the control and high-EDC diets were inseminated with semen obtained from control cocks and the remaining birds in each group with the semen of cocks on the high-EDC diet. In the second trial, the arrangement was reversed. Eggs were collected on days 2–7 after insemination and checked for the presence of embryos after incubation for 4 days. In a separate experiment, several hens from the control and two EDC-treated groups were inseminated with semen from control or EDC-treated cocks. The eggs were collected and incubated for 3 wk under standard conditions of temperature and humidity in a laboratory incubator.

Table 2. *Flock production rate during months 1–3 of laying by Leghorn hens fed EDC-fumigated mash*

Duration of laying period (months)	Flock production rate (%) for groups fed EDC levels (ppm) of 0	250	500
1	90·1	86·0	84·7
2	87·1	75·5	84·5
3	82·0	75·7	83·7

At the end of the 2-yr feeding period, blood samples were taken from all the hens for serum analyses (for methods, see Alumot *et al.* 1976).

All the results were subjected to analysis of variance, and the multiple range test of Duncan (1955) was used to evaluate treatment means.

RESULTS

Records of growth and feed efficiency during the first 8 wk of the trial (Table 1) showed no differences between the control and EDC-treated groups. Subsequently the chicks were weighed monthly, and at 4·5 months of age, when the sexes were separated, there was again no significant difference between treatments. The average female weights were 1376 ± 85, 1385 ± 77 and 1363 ± 68 g in the control, high-EDC and low-EDC groups, respectively, while the corresponding weights of the males were 1875 ± 108; 1820 ± 95 and 1820 ± 111 g.

The performance of the laying hens was not affected by EDC treatment during the first 3 months of full laying activity (Table 2).

A highly significant difference in egg weights between the control group and the two EDC-treated groups appeared in month 3 of full laying activity (Table 3) and persisted up to the end of the 2-yr trial (Table 4).

Table 3. *Mean weight of eggs from groups of Leghorn hens fed EDC-fumigated mash*

Duration of laying (wk)	Egg weights (g) for groups fed EDC levels (ppm) of 0	250	500
8	60·3	61·5	60·0
9	62·8	59·6	60·4
10	60·7	59·7	58·3
11	58·7	58·3	57·0
12	61·0	58·0*	57·3*

Values are means for eggs laid during each week by groups of 15 or 16 hens, and those marked with an asterisk differ significantly from the control value (*$P < 0·01$).

Table 4. *Mean egg weight determined individually twice each month on three consecutive days for groups of Leghorn hens fed EDC-fumigated mash*

Duration of laying (months)	Mean egg weights (g) for groups fed EDC levels (ppm) of		
	0	250	500
4	58·8 ± 3·2 (15)	55·7 ± 4·9 (16)	55·6 ± 4·1 (15)
7	63·8 ± 3·8 (15)	58·0 ± 5·4 (15)	55·7 ± 3·7 (14)
11	67·4 ± 5·0 (15)	61·1 ± 5·9 (14)	60·5 ± 4·8 (12)
12	66·9 ± 4·2 (14)	60·3 ± 5·2 (14)	60·3 ± 4·4 (12)
13	65·9 ± 4·8 (14)	60·8 ± 5·3 (14)	60·0 ± 4·1 (12)
14	66·1 ± 5·3 (14)	60·4 ± 5·4 (14)	59·1 ± 3·6 (12)
15	65·1 ± 4·9 (14)	59·6 ± 6·1 (14)	58·9 ± 3·1 (12)
16	64·5 ± 5·0 (14)	59·1 ± 6·7 (14)	57·8 ± 3·3 (12)
17	63·4 ± 5·7 (14)	57·9 ± 5·1 (14)	58·6 ± 3·8 (12)
18	64·3 ± 5·9 (14)	59·8 ± 5·8 (14)	58·4 ± 4·1 (12)

Values are expressed as the means ± SD for the numbers of hens indicated in parenthesis and all those for EDC-treated hens were significantly lower ($P < 0·01$) than the corresponding control figure.

The few deaths that occurred during the experiment (Table 4) were caused by leucosis (one hen from each group) at the beginning of the laying period and by reproductive disorders (a total of five hens) at periods indicated in the table.

From month 4 of laying, the performance of hens on the high-EDC diet, but not of those on the lower, dropped significantly ($P < 0·05$) in comparison with that of the control group (Table 5). The lower production rate of the hens fed 500 ppm EDC in the mash was caused by the lowering both of the individual laying rate and of the number of layers in the group.

The feed intake of the high-EDC group was lower than that of the other groups, particularly in the period after moulting was induced (Table 6). It is possible that the reduced intake was a consequence of the lower egg production.

In contrast to the females, the males were not affected by EDC treatment. No difference occurred between cocks of the control and EDC groups as far as semen production and sperm motility were concerned. The number of poor semen producers was similar in all groups (three or four/group). These cocks were not used in the fertility tests.

The results of the two fertility trials were as follows: control cocks and EDC hens, 100% fertile eggs; control cocks and control hens, 97%; EDC cocks and EDC hens, 100%; and control hens and EDC cocks, 87%. No significant differences were found in the fertilization rates following insemination with the control- or EDC-group semen, regardless of whether the hens were from the control or EDC group. Hatchability (% of fertile eggs) was 85·7, 94·1 and 96·8 in the control, low-EDC and high-EDC groups, respectively, re-

Table 5. *Egg-production by EDC-treated Leghorn hens from month 4 of laying to the end of the trial*

Duration of laying (months)	Flock production rate, %			Layers' production rate, %			Laying hens, % of total		
		EDC			EDC			EDC	
	Control	250 ppm	500 ppm	Control	250 ppm	500 ppm	Control	250 ppm	500 ppm
4	83·6	74·5	64·5*	83·6	74·5	69·0*	100	100	93
5	75·5	73·6	57·4*	75·5	73·6	66·2*	100	100	87
6	68·3	67·4	46·7*	68·3	67·4	59·4*	100	100	79
7	61·6	51·0*	50·0*	61·6	55·9	58·3	100	73	86
8†	49·3	36·0	21·3*	49·3	42·0	25·6*	100	86	83
10	21·0	27·7	8·0*	26·3	35·3	16·0*	80	79	50
11	63·1	54·3	47·1*	63·1	58·5	56·5	100	93	83
12	72·9	79·7	62·9*	78·1	79·7	68·6*	93	100	92
13	76·6	74·7	68·7	82·1	74·7	74·9	93	100	92
14	68·4	71·6	54·2*	73·3	71·6	59·1*	93	100	92
15	58·0	63·0	46·8*	62·5	63·0	57·7	93	100	83
16	59·0	60·8	48·4*	63·5	60·8	58·1	93	100	83
17	53·6	48·9	41·3*	57·7	57·3	49·6*	93	86	83
18	50·0	50·0	41·0*	63·6	58·3	55·2	79	86	75

†In month 8 (November 1972) all the hens were subjected to forced moulting by feed withdrawal in order to renew the production for the second year. No eggs were laid in December, and laying began again in January 1973.

Values marked with an asterisk differ significantly ($P < 0·05$) from the control, on the basis of analysis of the individual production rate.

Table 6. *Effect of EDC fumigation of mash on monthly feed consumption by laying hens*

Duration of laying (months)	Feed consumption (kg mash/hen/month) by groups fed EDC levels (ppm) of		
	0	250	500
3	2·9	2·6	2·8
4	3·5	3·3	3·3
5	2·8	2·7	2·8
6	2·6	2·3	2·3
7	2·8	2·7	2·7
8*	1·6	1·7	1·6
9	2·7	2·5	2·1
10	2·7	2·7	2·3
11	3·0	3·0	2·3
12	3·2	3·1	2·7
13	3·9	3·6	3·3
14	3·0	2·8	2·6
15	2·9	2·7	2·6
16	3·6	3·2	3·0
17	2·8	2·6	2·2
18	3·4	3·3	2·8
Total	47·4	44·8	41·4

*Food restricted to induce forced moulting (see footnote (†) to Table 5.

gardless of the semen source, and the respective average chick weight was 41·2, 40·0 and 39·6 g. The differences were not statistically significant.

At the end of the 2-yr trial, blood samples from all the hens were analysed for biochemical parameters of liver function and general health (Table 7). No significant differences were found between control and treated hens.

Table 7. *Terminal analyses of serum from laying hens fed EDC-fumigated mash for 2 yr*

Serum component	Mean values for groups fed EDC levels (ppm) of	
	0	500
Glucose (mg/100 ml)	220·7 ± 9·4	207·8 ± 7·2
Protein (g/100 ml)	6·1 ± 0·3	6·3 ± 0·4
Albumin (g/100 ml)	2·1 ± 0·13	2·2 ± 0·09
Globulin (g/100 ml)	4·0 ± 0·37	4·1 ± 0·35
Uric acid (mg/100 ml)	5·2 ± 0·5	5·3 ± 0·4
Cholesterol (mg/100 ml)	200·8 ± 21·5	240·0 ± 32·8
GOT (IU)	106·0 ± 6·3	105·3 ± 9·9
GPT (IU)	4·3 ± 1·1	4·2 ± 1·1

GOT = Glutamic–oxalacetic transaminase
GPT = Glutamic–pyruvic transaminase
Values are means ± SEM for groups of ten hens.

DISCUSSION

It is clear from the results reported here that neither EDC level fed in the mash influenced the growth or sexual development of male or female chicks. However, the 500-ppm level affected the feed intake and performance (number and weight of eggs) of the laying hens. At the lower level (250 ppm), egg weight but not egg number was significantly affected. Since the difference in egg weight between both EDC groups and the control was similar (about 5–6 g) and did not change during more than a year of laying, the effect of EDC on production may be regarded as relatively moderate. Ethylene dibromide, a chemically related compound, affected egg size drastically, causing a continuous drop in weight up to cessation of laying even at a level of 20 ppm in the mash (Bondi *et al.* 1955).

In contrast to the action of ethylene dibromide (Alumot, Nachtomi, Kempenich-Pinto, Mandel & Schindler, 1968), EDC did not affect the fertility of laying hens. The effect of the low EDC level was restricted to a moderate but constant depression in egg weight. To be on the safe side, therefore, we suggest that the tolerance for hens should be no more than 100 ppm EDC. The acceptable daily intake, based on a mash consumption of 100 g and a body weight of 2 kg is then about 5 mg/kg body weight. For growing chicks the tolerance and the acceptable daily intake are, as for rats, 250 ppm and 25 mg/kg body weight, respectively.

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