foreign substances; or, in other words, within hens all substances are handled the same way, but between hens there can be very large variations in metabolism, storage, etc. This is probably one of the main reasons for finding such large variations in residues between hens killed at the same age and the large variations between eggs sampled at different dates and probably laid by different hens.

ACKNOWLEDGMENT

We wish to thank L. P. van der Salm for his help in the animal experiment and E. Reinders for his help in the residue determinations.

Supplementary Material Available: A listing of concentrations of organochlorine pesticides in fat, eggs (on a whole egg and on a fat basis), and feces (12 pages). Ordering information is given on any current masthead page.

LITERATURE CITED

Cecil, H. C., Bitman, J., Fries, G. F., Harris, S. J., Lillie, R. J., *Poult. Sci.* **52**, 648–653 (1973).
Cummings, J. G., Eidelman, M., Turner, V., Reed, D., Zee, K. T., Cook, R. E., *J. Assoc. Off. Anal. Chem.* **50**, 418–425 (1967).
Cummings, J. G., Zee, K. T., Turner, V., Quinn, F., Cook, R. E., *J. Assoc. Off. Anal. Chem.* **49**, 354–364 (1966).
Greve, P. A., Grevenstuk, W. B. F., *Meded. Rijksfac. Landbouwwet. Gent.* **40**, 1115–1124 (1975).
Kan, C. A., Jonker-den Rooyen, J. C., *J. Agric. Food Chem.*, preceeding paper in this issue (1978).
Kan, C. A., Tuinstra, L. M. G. Th., *J. Agric. Food Chem.* **24**, 772–775 (1976a).
Kan, C. A., Tuinstra, L. M. G. Th., *J. Agric. Food Chem.* **24**, 775–778 (1976b).

Received for review March 28, 1977. Accepted August 26, 1977.

Second Laying Cycle Effects of a Mixture of Organochlorine Insecticides on Broiler Breeder Hens

Cornelis A. Kan* and Jenny C. Jonker-den Rooyen

During the second laying cycle of broiler breeder hens, a mixture of hexachlorobenzene, α-, β-, and γ-hexachlorocyclohexane, heptachlor, p,p'-DDT, and dieldrin was added at three different levels between 0.01 and 1 mg/kg to the food with a view to study performance, following a similar treatment during the first laying cycle. Mortality and number of eggs produced per hen present were unaffected even when combined over both laying periods. Egg weight, specific gravity of the egg, and shell thickness did not differ due to the treatments. Fertility and neonatal mortality showed no influence of the treatments, but the number of good quality chicks hatched from fertile eggs was lowered in the highest treatment group.

In a previous publication we have reported that low levels of organochlorine insecticides had no influence on the performance of broiler breeders during their first laying cycle (Kan and Tuinstra, 1976). There were, however, two reasons for extending the experiment to a second laying cycle after forced moulting: (1) Davison et al. (1970) have shown that during severe dietary restriction (which in practical situations is the causative factor for the forced moulting) levels of dieldrin in the food, which are normally harmless to laying hens, will have an adverse effect. (2) Cecil et al. (1973) demonstrated that pullets fed DDT had thicker shells than the control hens, while hens in their second laying cycle laid eggs which had significantly thinner shells. Therefore, at the end of the first laying cycle, the hens were subjected to a forced moulting procedure and after a production stop of 3 weeks kept for a second laying cycle of 30 weeks. The experimental conditions were identical with those in the first laying cycle (Kan and Tuinstra, 1976). Accumulation and depletion in fat and eggs will be discussed in the accompanying paper (Kan and Jonker-den Rooyen, 1978).

MATERIALS AND METHODS

The experimental scheme of four treatment groups, each consisting of two replicate pens, was maintained. Housing and management were also identical with those described for the first laying period (Kan and Tuinstra, 1976). The forced moulting (production stop) was induced at 69 weeks of age by food withdrawal during 6 days. Thereafter the hens received ground oats during 3 weeks (1 week ad libitum and 2 weeks 100 g per hen per day). Following the laying stop, the amount of food provided was gradually increased as can be seen from Table I.

Table I. Level of Average Food Consumption per Hen per Day[a]

Age, weeks	M.E.,[b] kcal	Age, weeks	M.E., Kcal
69-70		77-78	400
70-71	290	78-81	430
71-73	240	81-84	400
73-74	290	84-86	370
74-75	315	86-101	355
75-76	340	101-108	370
76-77	370		

[a] Food consumption in weeks 70-73 consisted of ground oats (ad libitum or restricted). Starting week 73-74, normal food was provided. After 97 weeks all hens received the control food. [b] M.E. = metabolizable energy.

At the beginning of the second laying cycle, the number of hens in each group was reduced by random selection to 84 in order to equalize the number of hens in each group. During the laying pause the cocks were housed separately (not moulted) and fed the same (restricted) amount of food. After 97 weeks of age all the groups received the

Spelderholt Institute for Poultry Research, Ministry of Agriculture and Fisheries, Beekbergen, The Netherlands.

0021-8561/78/1426-0470$01.00/0

Table II. Intended Concentrations (mg/kg) of Organochlorine Insecticides in the Experimental Diets and Maximum Allowable Concentrations (mg/kg) in Dutch Poultry Food for Laying Hens and Broilers (Regulations of the Commodity Board of Animal Food)

Insecticide	Group 1	Group 2	Group 3	Group 4	Max. allow. concn: Laying Hens	Max. allow. concn: Broilers
Hexachlorobenzene	0	0.01	0.05	0.10	0.025	0.025
α-HCH	0	0.05	0.25	0.50	0.05 } 0.3	0.08 } 0.5
β-HCH	0	0.10	0.50	1.00		
γ-HCH	0	0.05	0.25	0.50		
Heptachlor	0	0.025	0.125	0.25	0.03	0.03 (incl. epoxide)
p,p'-DDT	0	0.10	0.50	1.00	0.2	0.2 (total DDT)
Dieldrin	0	0.025	0.125	0.25	0.03	0.03 (incl. aldrin)

control food in order to study diminution of the residues of the organochlorine insecticides in fat and eggs. The experiment was terminated when the hens were 108 weeks old.

Dietary composition was identical with that during the first laying period. The added concentrations of organochlorine insecticides and, for comparison, the maximum allowable concentrations in food are given in Table II. At regular intervals, especially during the depletion period, hens were killed for residue analysis.

At the age of 78 and 95 weeks, weight, specific gravity, and shell thickness of the eggs were determined as described previously (Kan and Tuinstra, 1976). Hatching eggs were collected once during a fortnight for determining hatchability and liveability of the chickens. A second planned hatch had to be cancelled because of a too low fertility rate. All birds which died were autopsied to determine the cause of death. Statistical analyses were made by analysis of variance according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Mortality. During the laying pause only one hen (out of ca. 350 hens) belonging to the control group died. There was, therefore, no indication of any adverse effect of starving during 6 days. The mortality due to diseases during the second laying cycle was 2, 5, 5, and 5 hens for group 1–4, respectively. Neither the number nor the causes of death revealed increased mortality due to the addition of increasing concentrations of organochlorine insecticides to the basic diet.

Productivity. After 6 days of starving, egg production has decreased below 10%. During the laying pause some hens apparently continued egg laying as still 2–3 eggs were collected daily per group. Following the return to normal food, egg production gradually increased and reached a maximum of about 65% during weeks 79–80 (6 weeks after the beginning of the second laying period). Afterwards there was a decline to an egg production of about 50% at 97 weeks of age, when the depletion period started. The total number of eggs laid per hen present, calculated biweekly, during the period of 73–97 weeks was 87.8, 87.1, 87.0, and 78.2 eggs for groups 1–4, respectively. (For more details, see Supplementary Material.) Peak production was, as usual, considerably lower than in the first laying period (Kan and Tuinstra, 1976). The total number of eggs produced indicates that the hens in group 4 (receiving the highest dose of organochlorine insecticides) might have been influenced by the treatment. However, the differences in the second laying period, as well as those for the combined laying periods, were not statistically significant ($P > 0.05$).

Egg Weight, Specific Gravity, and Shell Thickness. The egg weight, specific gravity of the egg and egg shell thickness of some 160 eggs in each treatment group were determined at the age of 78 and 95 weeks. The results are listed in Table III. Statistically significant differences in specific gravity and shell thickness, which occurred at 78 weeks, are indicated in the table. As in the first laying period, dose/response related thinning of the egg shell due to the imposed treatments could not be observed.

Table III. Egg Weight, Specific Gravity, and Shell Thickness[a]

Group no.	Egg wt, g	Specific gravity	Shell thickness, mm
	78 weeks		
1	67.03a	1.082a	0.346a
2	66.64a	1.083ab	0.350ab
3	66.32a	1.084b	0.358c
4	67.54a	1.082a	0.356bc
	95 weeks		
1	68.77a	1.078a	0.345a
2	68.15a	1.080a	0.356a
3	67.45a	1.080a	0.348a
4	69.25a	1.079a	0.353a

[a] Figures in one row followed by the same letter do not differ significantly ($P > 0.05$).

Table IV. Fertility and Hatchability

Group no.	No. of eggs set	No. of fertile eggs	No. of chicks hatched	No. of chicks hatched: As % of eggs set	No. of chicks hatched: As % of fertile eggs
1	543	476	412	75.9	86.6
2	544	497	426	78.3	85.7
3	554	412	356	64.3	86.4
4	523	478	382	73.0	79.9

Fertility, Hatchability, and Neonatal Mortality. The result of the hatching experiment is given in Table IV. As indicated in the Materials and Methods section, a second hatch had to be cancelled because of the too low fertility rate. The number of good quality chicks hatched as percentage of eggs set, revealed no difference between groups. However, in the number of good quality chicks hatched as percentage of fertile eggs, a significant difference ($P < 0.05$) was found between group 4 and the other groups. This difference was mainly due to the number of hatched chicks of inferior quality in this group, which had to be killed. There is not a clear explanation for this effect. Although there are some differences between groups and sexes, the number of deaths during early life was so small (approximately 1.5% during the first 2 weeks of life) that no statistical differences in mortality due to the treatment of the parents could be observed.

General. Starving of the broiler breeder hens during several days, induced a laying stop and a gradual renewal of the feathers. The dietary restriction did not cause an increase in deaths, which might have been expected. Also during the second laying period, there was no dose-related effects on egg shell quality as found by Cecil et al. (1973) using DDT.

Egg production was not influenced significantly as a result of the treatments. The only effect observed was a decline in the number of good quality chicks hatched from the fertile eggs of group 4.

In summary, addition of a low level mixture of organochlorine insecticides for a period of over 2 years to broiler breeders food had only a very marginal effect on the performance of these animals.

ACKNOWLEDGMENT

We wish to thank L. P. van der Salm for his excellent help in the animal experiment.

Supplementary Material Available: A listing of subgroup figures (4 pages) on egg production, egg shell quality and hatchability. Ordering information is given on any masthead page.

LITERATURE CITED

Cecil, H. C., Bitman, J., Harris, S. J., Lillie, R. J., *Poult. Sci.* **52**, 648–653 (1973).

Davison, K. L., Sell, J. L., Rose, R. J., *Bull. Environ. Contam. Toxicol.* **5**, 493–501 (1970).

Kan, C. A., Jonker-den Rooyen, J. C., *J. Agric. Food Chem.*, following paper in this issue (1978).

Kan, C. A., Tuinstra, L. M. G. Th., *J. Agric. Food Chem.* **24**, 772–775 (1976).

Snedecor, G. W., Cochran, W. G., "Statistical Methods", 6th ed, Iowa State University Press, Ames, Iowa, 1967.

Received for review March 28, 1977. Accepted August 26, 1977.

A Rapid and Simple Method for the Determination of Residues of 2-Chloroethylphosphonic Acid (Ethephon) in Tomatoes, Cherries, and Apples

Jakob Hurter,* Marianne Manser, and Bernhard Zimmerli

A rapid method has been developed for the detection of residues of 2-chloroethylphosphonic acid (ethephon) in tomatoes, cherries, and apples based upon degradation to ethylene at high pH values. The hormone released is determined by gas–solid chromatography. Extraction and purification of 2-chloroethylphosphonic acid from fruit tissue as described in earlier procedures can be omitted. Residue results are consistent with the direct identification of methylated ethephon by gas–liquid chromatography with a flame photometric detector. After a characteristic increase, maximum residues in field-treated crops were found to be 6.8 ppm on tomatoes, 4.1 ppm on cherries, and 1.2 ppm on apples 2 to 8 days after application. The natural content of ethylene in fruit is less than 5% of what can be expected from residues of ethephon.

Since the biological action of ethylene on the development of plants was reported (Neljubow, 1901), a number of physiological responses have been described and reviewed (Abeles, 1973). Fruit growers were unable to take advantage of these responses because of the difficulties in applying gaseous compounds to plants in the field. The search for nongaseous ethylene-releasing chemicals (Gowing and Leeper, 1955) was of little success until 2-chloroethylphosphonic acid (ethephon), described already 17 years earlier (Kabachnik, 1946), was found to be most effective (Bukovac, 1969; Maynard, 1963). Its excellent biological action, especially on the process of fruit ripening, has made it one of the most important plant growth regulators for agricultural purposes. Unfortunately, long-term feeding tests on dog and rat indicate a considerable inhibition of cholinesterases in blood plasma and erythrocytes, as it is known by a number of esters of phosphoric acid used as insecticides. Harmful accumulation of inhibitors of this kind in food can be circumvented by well-considered applications based on residue analysis. The gas chromatographic procedures developed earlier for residue analysis (Amchem Products Inc., 1971; Bache, 1970) determine the methylated phosphonic acid compound by GLC with a flame photometric or alkaliflame ionization detector, respectively. Even simplified extraction and clean-up procedures in recently published papers (Cochrane et al., 1976; Ernst and Anderegg, 1976) are rather time consuming.

In this paper, we described a rapid method suitable for routine analysis of ethephon residues in tomatoes, cherries, and apples, based upon the quantity of ethylene released from ethephon at pH values of 12–14, thus omitting tedious extraction and clean-up steps. This principle has already been successfully applied as a screening method for ethephon residues (Zimmerli, 1974).

MATERIALS AND METHODS

Material. Ethylene (99.5% pure, compressed to 1200 psi in tanks of 0.44 L) was supplied by Merck-Schuchardt, Munich. Ethephon (87% pure, technical grade) was a gift from Amchem Products Inc., Ambler, Pa. Activated alumina F-1 was obtained from Applied Science Laboratories Inc., State College, Pa. Acetone of analytical grade was distilled to remove volatile compounds interfering with the gas chromatographic procedure.

Standard Solution. Ethephon at concentrations of 10–100 mg/L of distilled water was kept in polyethylene bottles and renewed monthly. Ethylene of the tank was trapped in serum bottles and further diluted to 10.0, 1.0, and 0.1 ppm (v/v) in similar bottles containing N_2.

Treatment and Sampling of Fruit. Twenty-four tomato plants of the variety "Montfavet H 63-5" were sprayed by a hand gun with a solution of 0.2% of ETHREL, corresponding to a concentration of 960 ppm of ethephon. (ETHREL is the registered trademark of Amchem Products Inc. for this plant growth regulator

Swiss Federal Research Station Wädenswil, CH-8820 Wädenswil, Switzerland (J.H., M.M.) and the Federal Office of Public Health, Division of Food Control, CH-3001 Bern, Switzerland (B.Z.).

0021-8561/78/1426-0472$01.00/0