Bull. Environm. Contam. Toxicol. 20, 111-119 (1978)

Reproductive Success of Hens and Cockerels Originating from Eggs Sprayed with 2,4-D, 2,4-5-T and Picloram Followed by Early Performance of Their Progeny after a Comparable *In ovo* Exposure[1]

J. D. Somers, E. T. Moran, Jr., and B. S. Reinhart

Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada

In addition to dramatic reductions in embryonic survival rates of pheasant and partridge eggs following an external exposure to 2,4-D, LUTZ-OSTERTAG and LUTZ (1970) noted important inadequacies in gonadogenesis such that partial or total sterility would be inevitable. Other studies on the effects of phenoxyacetic acid herbicides on the in ovo development of avian eggs have proved negative (SOLOMON et al., 1973; SOMERS et al., 1974 a, b,c; GROLLEAU et al., 1974); however, none investigated progeny effects beyond 4 weeks of age and subsequent possible consequences on reproductive capacity.

The present study further considers the reproductive performance of progeny from hens' eggs sprayed with 2,4-D, 2,4-5-T and picloram each at three stages of embryonic development as previously described by SOMERS et al. (1977). Fertile eggs as derived from these same hens and cockerels were further contaminated followed by an assessment on hatching success as well as early chick performance.

Materials and Methods

Cockerels and hens were reared sex separate in floor pens on a common growing feed from 4 to 20 and 16 weeks of age, respectively. Wing banding permitted identification of birds corresponding to the original egg treatments: 1) control groups to separate the effects of transportation to and from the incubator as well as spraying with water and a formulation blank of herbicide inert ingredients; 2) spray contamination with 2,4-D, 2,4-5-T and picloram; and 3) each of the germ ages at

[1] The data contained herein were excerpted from a Ph.D. thesis submitted to the University (1975) by the senior author (J.D.S.).

0007-4861/78/0020-0111 $01.80

0,4 and 18 days incubation when treated for both the control and herbicide groups. Performance of the females among the 3 control groups as measured by weight gain and mortality during the 12 week rearing period was statistically shown to be comparable. Consequently, in order to allow for a genetic control the group of birds used to evaluate the effects of egg transportation was replaced with same age commercial source Single Comb White Leghorn strain hens that were original employed.

At 16 weeks of age the females were individually caged and fed the same laying ration. Males were continued in the same floor pens, however, their feed was not changed to correspond with the females until 20 weeks of age. Egg laying was monitored over three 28 day periods from 41 to 53 weeks of age when egg formation rate was expected to be at its maximum. During the last two periods artificial insemination was performed weekly. Reproductive success was in turn ascertained from two separate incubation studies using the so-derived eggs. In both cases, hens originating from eggs subjected to each spray treatment and the three stages of embryonic development when contaminated were inseminated with pooled semen from comparably treated cockerels. Hens of the genetic control received sperm from cockerels of the transportation control group.

Sperm counts were estimated at the weekly inseminations by combining 100 µl of pooled semen with 5 ml of normal saline and comparing the optical density at 525 mµ to a known concentration of standard boar sperm. Testes were removed at 55 weeks of age for gross appearance and weighing.

Each of the incubation studies utilized 20 successive days egg collection. While the first required no further manipulation prior to or during incubation, the second necessitated that the eggs be subjected to the same control or herbicide treatments as both parents had been exposed only application was restricted to 0 day embryonic development. Procedures and/or conditions of egg stage, spraying and incubation were as described previously (SOMERS et al., 1974 c).

Chicks resulting from both incubation studies were vent-sexed immediate to hatching and subsequently reared in pens of an electrically heated raised wire floor brooder battery to 4 weeks of age. All chicks received the same commercially available chick starter and water ad libitum. Ten chicks of each sex were represented from each of the 4 successive 5 day intervals that eggs were stored prior to the onset of incubation for all of the primary variables (spraying and stage of embryonic development when contaminated).

Over the period of one generation and into the second, not one of the parameters of evaluation used in the present investigation clearly indicated that 2,4-D, 2,4,5-T or picloram when used at 10x recommended dosage adversely affected normal development of Gallus domesticus. Similarly, HILBIG et al. (1976) spraying 2,4-D on Japanese Quail eggs prior to incubation also failed to observe any alteration in subsequent reproductive capacity of offspring or their hatching success. Both would refute the contention of LUTZ-OSTERTAG and DIDIER(1971) that avian embryos exposed to the phenoxyacetic acids underwent a feminization of physiological castration.

Summary

Hens and cockerels that came from fertile eggs which were subjected to spray contamination with a 10x recommendation dosage of 2,4-D, 2,4,5-T and picloram either prior to incubation or after 4 and 18 days embryonic development were evaluated for various aspects of reproduction.

Capacity of the hen was assessed in terms of egg production from 41 to 53 weeks of age. During the last 8 weeks egg weight as well as shell porosity and strength, were measured. Over this same period of time sperm counts were estimated from weekly collections for the cockerels and terminated with evaluating testis gross appearance and determining their weight.

Pooled semen from these same cockerels was used to artificially inseminate hens which originated from the identical earlier egg treatment. Two incubation studies were performed with the subsequent eggs. The first evaluated reproductive success as a function of parental treatments while the second involved an egg retreatment with spray contamination restricted to the pre-incubation period. In both cases the proportion of viable germs resulting from these matings and occurrence of late embryonic mortality, as well as malformations, were monitored. Measurement of weight gain and deaths of resulting chicks 0 to 4 weeks of age finalized the experiment.

All data were statistically examined to evaluate the effect due to egg spraying, embryonic age upon contamination and their interactions. In every instance neither was there any effect attributable to extent of germ development when treated nor an interaction involving the herbicides to the consequences of contamination than anyother. Significant effects due to spray treatment occurred with several parameters, however, one or more of the controls was assessed to be either in the poorer or equivalent position relative to any herbicide treatments. In general, there was no definitive evidence that 2,4-D, 2,4,5-T and picloram had any repercussion of domestic fowl reproduction through one generation and into the second.

TABLE 1

Production and Performance of Hens Derived From Eggs Sprayed with Commercial Source Herbicide[a]

Treatment	Production % H.D.B.[b]	Egg Wt. g[c]	Shell Porosity %Δ Egg Wt.[d]	Shell Strength μ[e]
Controls				
Genetic	86.2[y]	59.3	4.7	24.7
Water Spray	79.0[z]	57.3	4.3	25.8
Formulation Blank	78.3[z]	58.1	3.5	26.6
Herbicides				
2,4-D	80.7[z]	58.5	4.5	26.9
2,4,5-T	77.3[z]	59.9	4.4	26.6
Picloram	78.6[z]	59.4	4.5	27.6
σ(144 df)	6.4	3.5	0.8	2.9

[a]Spraying was applied at the rate of 746 l/ha. Available preparations of 2,4-D ("Esteron 99"), 2,4,5-T ("Esteron 245") and picloram ("Tordon 22K") were all diluted in relation to this volume to supply 10x the normal amounts used in practice (ca. 11.2 kg/ha) with the formulation blank supplying corresponding quantities of non-active ingredients.

[b]Production mean, 41-53 weeks of age on a hen day basis. Superscript letters refer to Duncan's Multiple Range Test with figures having common letters not being significantly different ($P > 0.05$).

[c]Means based on 5400 total eggs over the period of 45-53 weeks of age.

[d]Shell porosity was estimated from the loss in weight after a 10 day storage period as measured on a total of 1448 eggs over 45-53 weeks of age.

[e]Shell strength was estimated by its deformation from normal shape when a 500 g weight was placed on its equator. Expressed as microns with larger values implying weaker shells. Means based on 1448 total eggs collected over 45-53 weeks of age.

Results and Discussion

Egg production and several parameters to evaluate shell quality were monitored with respect to the effects due to spraying the egg of hen origin as well as the embryonic age when contamination occurred. An analysis of variance of this factorially designed experiment indicated neither embryonic age of contact nor its interaction with any of the spray treatments proved significant ($P > 0.05$) for these measurements. The data shown on Table 1 are orthogonal comparisons involving spray treatments where the only significant alteration appeared. A partitioning of the effect due to spraying revealed that the rate of egg production with the commercial source hen serving as genetic control was better than all birds originating with the experimental cross. Weight of egg produced and estimates of shell porosity as well as strength were not measurably influenced by any of the variables in question. An overall feed efficiency of 2.07 g of feed consumed per g of egg produced was marginally better than encountered in normal practice and consistent with all treatments.

The results obtained with cockerels were similar to those observed with the same age of hens. There were no apparent effects at adulthood when the egg of origin was sprayed at various stages of embryonic development or were there any significant interactions with any spray treatment ($P > 0.05$). There was, however, a detectable herbicide influence independent of the other variables. In this respect, a partitioning of means revealed that semen from 2,4,5-T contaminated cockerels had a lower sperm count than observed with either the control group or encountered with the other herbicide treatments (Table 2). In all cases there were no significant differences in testes weight, no visually apparent physical abnormalities or differential in the left and right testes.

TABLE 2
Sperm Count and Testes Weight of Cockerels Derived from Eggs Sprayed with Herbicides

Treatment	Sperm Count 1×10^6/cc[a]	Testes Weight g[b]
Controls		
Transportation	2120[y]	17.8
Water Spray	1976[y]	21.1
Blank Spray	2010[y]	21.7
Herbicides		
2,4-D	1992[y]	20.7
2,4,5-T	1706[z]	23.1
Picloram	1950[y]	23.9
σ(df)	265(60)	7.5(68)

[a]Means of 7 weekly collections from 48 to 55 weeks of age. Superscript letters in this column refer to Duncan's Multiple Range Test. Figures with common letters are not significantly different ($P > 0.05$).

[b]Mean of right plus left testis from 55 week old cockerels.

TABLE 3

Incubation Performance of Eggs from Hens and Fertilized Through Cockerels Both Originating from Eggs Sprayed with Common Herbicides and the Influence of a Second Year's Retreatment[a]

Treatment	% VG of Total Eggs Incubated[c]		% of Viable Germs (VG)[b]							
			EDG		LDG		PDG		Hatch	
	No-T[d]	Re-T[d]	No-T	Re-T	No-T	Re-T	No-T	Re-T	No-T	Re-T
Controls										
Genetic	92.1^{y}	87.1^{y}	5.2^{x}	14.6^{xy}	2.4	8.2^{x}	2.7	2.2	89.7	75.0^{x}
Water Spray	92.9^{y}	84.1^{x}	7.1^{x}	11.7^{x}	2.7	4.6^{yz}	0.2	1.9	90.0	81.8^{yz}
Blank Spray	92.8^{y}	91.6^{yz}	8.7^{xy}	12.3^{x}	1.3	6.7^{xy}	1.7	1.5	88.4	78.7^{xyz}
Herbicides										
2,4-D	93.8^{y}	87.6^{y}	11.9^{y}	17.5^{y}	3.0	3.6^{yz}	0.8	1.7	84.3	77.2^{xy}
2,4-5-T	85.9^{z}	92.9^{z}	9.5^{xy}	11.4^{x}	2.9	3.4^{yz}	1.2	1.6	83.1	82.7^{yz}
Picloram	94.8^{y}	92.4^{z}	7.8^{x}	9.3^{x}	2.1	2.8^{z}	1.7	2.5	88.4	85.4^{z}
σ(360 df)	7.3	7.5	7.1	9.7	2.4	5.1	1.5	2.5	10.4	13.4

[a] The superscripted letters in any data columns refer to Duncan's Multiple Range Test. Figures with common letters are not significantly different ($P < 0.05$).

[b] VG-viable germs; EDG=early dead germs (deaths 6 days or earlier); LDG=late dead germs (deaths after 6 days not breaking shell); PDG=pipped dead germs (deaths after breaking shell but not hatched).

[c] % viable germs of total eggs set for incubation were ascertained from gross appearance of broken-out EDG.

[d] No-T = no treatment of eggs prior to incubation while Re-T = retreatment and entailed a second year's spraying with the same aqueous suspension. The genetic control was untouched in both cases.

A further examination of the sperm count data showed that the first two pooled semen collections out of cockerels originating from eggs sprayed with 2,4,5-T prior to incubation had unusually low values. Collections subsequent to this time, however, assumed counts comparable to all other treatments. Attesting to the inadequacy in fertilizing capacity of these early collections is the reduction of viable germs from similarly treated inseminated hens (Table 3). Likewise, the larger than average proportion of viable germs at a later time when eggs were further contaminated by an additional exposure to 2,4,5-T prior to incubation indicated that the condition was corrected and probably not related to herbicide presence per se.

The data relative to viable germ performance through 21 days incubation (Table 3), occurrence of malformations and chick mortality (Table 4) together with early live performance (Table 5) all parallel the statistical evaluations as existed with initial egg contact (SOMERS et al., 1977) and subsequent adult performance as given earlier (Tables 1 and 2). No effects could be

TABLE 4
Occurrence of Embryonic Malformations and Early Mortality of Chicks Derived from Hens and Cockerels Both Originating with Herbicide Sprayed Eggs and the Influence of a Second Year's Retreatment

Treatment	Malform., % of VG-EDG[a]		% 0-4 Wk. Mortality	
	No-T[b]	Re-T[b]	No-T	Re-T[c]
Controls				
Genetic	1.1	2.0	2.9	1.9[yz]
Water Spray	0.3	1.5	1.7	0.0[y]
Blank Spray	0.6	1.8	2.5	0.8[y]
Herbicides				
2,4-D	1.0	0.7	1.7	3.3[z]
2,4-5-T	1.6	1.2	0.8	2.5[yz]
Picloram	1.0	0.8	1.7	0.8[y]
σ(30df)	0.7	1.1	0.7	1.1

[a]All malformations were located in the LDG and PDG groups and expressed as a % of VG less the EDG component where gross abnormalities could not be ascertained.
[b]See footnote d, Table 3.
[c]See footnote a, Table 3.

detected due to the embryonic age when contaminated. There were also no interactions of embryonic age at contact with spraying or herbicide treatments to indicate that the germ is more liable to contamination at one time than another.

When considered as the primary variable, responses due to spray treatments were observed; however, one or more of the control groups was in the poorer position for each

parameter when the means were partitioned and evaluated. The proportion of total hatched eggs with both studies was within the realm of expectation and there were no differences in the proportion of malformations encountered. What malformation appeared were restricted to late germs and typical of aberations encountered in normal practice.

TABLE 5

Live Performance of Chicks as Derived From Hens and Cockerels Both Originating From Herbicide Sprayed Eggs and the Influence of a Second Year's Retreatment, Weight Gain 0-4 Weeks of Age,g.

Treatment	Males		Females	
	No-T[a]	Re-T[a]	No-T	Re-T
Controls				
Genetic	273^{x}	293^{x}	229	244
Water Spray	266^{yz}	303^{y}	235	244
Blank Spray	268^{xy}	299^{xy}	234	243
Herbicides				
2,4-D	268^{xy}	303^{y}	231	242
2,4,5-T	262^{yz}	292^{x}	227	244
Picloram	260^{z}	291^{x}	229	245
σ(df)	27(638)	33(641)	24(633)	28(626)

[a]See footnotes a and d Table 3.

Rearing of second generation chicks through the crucial early weeks of development failed to disclose any consequences which could be related to either the original or second year herbicide exposure. Mortality occurring with the 2,4-D and 2,4,5-T retreated males was higher than observed with controls that relied on chicks of comparable parent origin but it was no different from the death rate found with genetic controls. Females failed to show any differences in mortality attributable to treatment. The better gains with chicks from the incubation study entailing egg retreatment over the earlier one where no spraying was involved is not unexpected nor would be the converse. Each time feedstuffs and environment conditions were different with both having extensive influence on early growth.

It is particularly important to note that the present study relied on relatively coarse parameters by which to judge the consequences of herbicides on avian development. Perhaps the use of more sophisticated means of evaluation would have detected alterations attributable to these common farm compounds. However, one might have expected any of the more serious changes to ultimately surface in a more obvious form. Administration of estrogens to the avian embryo in minute quantities can dramatically alter gonadogenesis to the extent that repercussions on adult reproduction are readily apparent (WENTWORTH et al., 1968; PANTIĆ and KOSANOVIĆ, 1973).

References

GROLLEAU, G., E. de LAUAUR and G. SIOU: Ann. Zool.- Écol. Anim. 6, 313 (1974).

HILBIG, V., K. LUCAS, V. SEBEK and H. MÜNCHOW: Anz. Schädlingkde., Phlanzenschutz, Umweltshutz 49, 65 (1976).

LUTZ-OSTERTAG, Y. and R. DIDIER: C.R. Soc. Biol. 165, 2364 (1971).

LUTZ-OSTERTAG, Y. and H. LUTZ: C.R. Acad. Sci. Paris 271 (Serie D), 2418 (1970).

PANTIĆ, V. R. and M. V. KOSANOVIĆ: Gen. Comp. Endocrinol. 21, 108 (1973).

SOLOMON, K. E., R. E. DAHLGREN and R. L. LINDER: Proc. S.D. Acad. Sci. 52, 95 (1973).

SOMERS, J. D., E. T. MORAN, JR. and B. S. REINHART: Bull. Environ. Cont. Toxicol. 11, 339 (1974 a).

SOMERS, J. D., E. T. MORAN, JR. and B. S. REINHART: Bull. Environ. Cont. Toxicol. 11, 511 (1974 b).

SOMERS, J. D., E. T. MORAN, JR., B. S. REINHART and G. R. STEPHENSON: Bull Environ. Cont. Toxicol. 11, 33 (1974 c).

SOMERS, J. D., E. T. MORAN, JR. and B. S. REINHART: Bull. Environ. Cont. Toxicol. (Submitted for review, 1977).

WENTWORTH, B. C., B. G. HENDRICKS and J. STURDEVANT: J. Wildl. Mgmt. 32, 879 (1968).