THE EFFECTS OF AN ORGANIC PHOSPHATE COMPOUND (BAYER 21/199) ON THE SEMEN QUALITY OF BULLS

by

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INTRODUCTION

Livestock owners have suffered losses estimated in excess of 100 million dollars annually as a result of the cattle grubs, Hypoderma bovis and Hypoderma lineatum. If additional damage resulting from flies, screw worms, lice, mange, and tick infestations is considered, the figure may be over 200 million dollars per year. Much of this loss is due to hide damage, reduced gains in weight, and decreased milk production.

The organic phosphate compounds have been studied extensively in the last few years as aids in controlling these various parasite problems. The popularity of these compounds has been due to the decreased residue as compared to that of chlorinated hydrocarbons. Another reason for their increased use is that many insects resistant to the chlorinated hydrocarbons are readily destroyed by organic phosphate insecticides.

Control of cattle grubs and other parasites has been seriously hampered because treatment required individual handling and application of insecticidal agents to animals. This form of treatment is impractical if not impossible in large herds. To overcome these undesirable features an organic phosphorus compound, Bayer 21/199 (O-(3-chloro-4-methylumbelliferone O,O-diethyl phosphorothioate)[1] has been developed. This product may be applied with a sprayer which permits treatment of large numbers of cattle efficiently in a short time. At present this compound is the only insecticide that can be applied by spraying to control cattle grubs systematically.

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[1] Hereafter referred to as Bayer 21/199.

Poisoning of domestic animals has been recognized as a result of contact with organic phosphates and there has been intensive investigation into the toxicity of these compounds. No references to the effects of organic phosphates on the semen quality in bulls could be found in the literature.

The investigation of sterility after spraying with Bayer 21/199 was suggested by a reported field case of sterility in bulls that had been sprayed with this compound.

REVIEW OF LITERATURE

Lagerlöf (8) stated that disturbances in spermatogenesis occurred chiefly in association with the following conditions: (a) Testicular hypoplasia: in this condition, the bulls were usually aspermatic but had fully developed sexual desires; in other cases certain portions of the testicle developed in such a manner that spermatozoa might develop but were fewer in number than normal. (b) Degenerative changes in the epithelium of the seminiferous tubules: this was the most common cause of disturbances in spermatogenesis. Such degeneration occurred from a multitude of causes such as hormonal disturbances, toxic influences or thermic changes. Roberts (16) divided the causes of degeneration into physical, traumatic, infectious, nutritional, genetic, and hormonal. (c) Inflammatory changes in the testicle or epididymis were usually the result of infections with Brucella abortus, Corynebacterium pyogenes, or Actinomyces bovis.

Certain chemicals have been proven to decrease spermatogenesis and others have been suspected as causes of reduced fertility. In experimental work conducted by Vlahos et al. (18) it was demonstrated that in bulls

experimentally poisoned with highly chlorinated napthalenes, the semen quality was severely disturbed but returned to normal in approximately ten months. Roberts (16) stated that seminal degeneration occurred in rams following dipping in arsenic solutions. Iodides were suggested as a possible cause of disturbances by Roberts[1] but this has not been confirmed. Research by Becze (1) indicated there was little alteration in semen quality of bulls and rams when bathed in and fed solutions of BHC and DDT. The intramuscular injection of carbon tetrachloride failed to produce changes. It has been suggested by Karczmar[2] that organic phosphates may have a lethal effect on the fetus.

Radeleff et al. (11) stated that an insecticide for use against parasites must meet several specifications. It should be effective against the parasite but it must not harm the host when used as directed. It should not cause injury if treatments are repeated frequently over long periods of time. It should have a safety margin even though handled somewhat carelessly. The ideal insecticide should not be stored in the body.

A new field of organic phosphorus insecticides was opened during the Second World War by Gerhard Schrader, a German chemist, engaged primarily in the search for more powerful agents of chemical warfare. Following World War II, American scientists discovered records of these highly toxic products. Since that time these chemicals have been subjected to intense study and extensive experimentation. A variety of compounds have been developed by chemical linkage of phosphorus with sulfur and other elements.

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[1] Stephen J. Roberts, personal communication, 1959.

[2] Alexander G. Karczmar, personal communication, 1959.

The development of resistance by insects to chlorinated hydrocarbon insecticides has stimulated research. It has been found that the inclusion of organic phosphates in orchard and garden sprays, livestock sprays and dips and insect powders has increased their effectiveness against resistant insects.

Many of the newer organic phosphorus compounds are less toxic for livestock than the older ones. These preparations have gained widespread use among livestock owners in an effort to control cattle grubs, screw worms, horn flies, mange mites, lice, and ticks in cattle, horses, swine, sheep, and goats.

Toxicology

All organic phosphate compounds are cholinesterase inhibitors or anticholinesterases. These compounds are frequently referred to as nervous system poisons or "nerve gases".

There is a wide variation of activity in compounds that inhibit cholinesterase. Radeleff et al. (13) suggested that results of experiments may be widely affected by the presence of other closely related compounds as impurities. Occasionally, poisonings follow prescribed dosages since human error permits the inclusion of chemically impure substances into any manufactured product. Another factor affecting results in producing experimental poisonings is the recognized variation in individual animal susceptibility. In organic phosphate poisonings, depletion of cholinesterase reserves affects the susceptibility. Prolonged exposure to minute quantities of organic phosphates may reduce this reserve to a point where a small additional dose would produce a serious

toxicity. An example would be animals grazing near pastures or fields that are frequently treated with organic phosphate compounds.

Jolly (6) explained the general toxicology of the organic phosphates as follows:

> "Acetylcholine is formed at the end of many nerve fibres of the central nervous system, the autonomic nervous system and somatic motor nerves. The nerve impulses cause release of acetylcholine by which it is able to cross the synaptic junction. This action of acetylcholine is very brief due to its almost immediate destruction by the enzyme cholinesterase. The organic phosphorus insecticides inactivate cholinesterase with the result that acetylcholine accumulates in abnormally high concentrations at the sites of liberation and causes over stimulation of the target cells."

Jolly (6) and Radeleff and Woodward (14) stated that blood cholinesterase levels may be used as a guide or an indication of the degree of organic phosphate intoxication. There is a wide variation in what may be termed normal cholinesterase levels in different animal species or individual animals within the same species. It is generally agreed that a normal level for an animal may be established only if several samples are obtained before exposure to the organic phosphate preparation. Most workers accept a fall of thirty to forty percent of cholinesterase blood levels as insignificant. Jolly (6) reported that a fall in excess of sixty percent has been observed to be dangerous while others suggested eighty percent to be the danger mark. Radeleff and Woodward (14) stated that animals may have a relatively high cholinesterase level while showing no apparent signs of poisoning. The opposite situation may exist in which an animal showing no apparent signs of poisoning will have a near zero reading.

Table 1 is a partial reproduction of a summary of results of spray and dip treatments of cattle, sheep and goats with various organic phosphate

insecticides. This work was done by Radeleff and Woodward (14).

Table 1. Toxicity of some organic phosphorus compounds.

Chemical	Animal	Percent of insecticide in spray or dip		
		Lethal	Toxic	Non-toxic
Parathion	Calves	0.02	0.01	---
	Sheep	1.00	---	---
	Goats	1.00	---	0.10
EPN	Calves	0.25	0.05	0.025
Diazanon	Calves	0.25	0.10	0.05
Bayer 21/199	Calves	0.75	0.50	0.25
	Cattle	---	---	2.00

These same authors (13) cited one instance in which a yearling Hereford heifer was given 50 mg/kg of compound Bayer 21/199 and died of poisoning; a yearling heifer given 25 mg/kg was unaffected.

Bayer 21/199 has the unusual property of acting systemically to control some insects when applied as an external spray. In preliminary studies using P^{32} labeled preparations this product was found to be poorly absorbed through the skin and slowly eliminated. Most of the small amount absorbed was metabolized to other compounds (Lindquist 9).

In later studies, Robbins et al. (15) using P^{32} labeled Bayer 21/199, found that only low levels of radioactive compound were present in the blood of two Hereford bulls after spray application of the compound. Further suggestion of poor absorption of the compound was indicated by the small amount, (about 2.4 percent suspension and 6.3 percent of an emulsion) of the applied dose, in the urine of the two animals two weeks after treatment. At that time only low levels of organosoluble compounds which

behaved like 21/199 were present in the tissues but a considerable residue of 21/199 was present externally.

Symptoms

Smith and Jones (17) stated that symptoms of poisoning usually developed within an hour or two after contact with a toxic dose of organic phosphates. This contact might be the result of inhalation of vapors, absorption through the skin or ingestion. Radeleff (12) reported deaths in cattle within five minutes after spraying with TEPP.

Most workers observed the first symptom of a toxic dosage in cattle to be a profuse salivation that approached the consistency of water. Animals breathed with their mouths held partially open and with greatly exaggerated respiratory movements. As respiratory symptoms increased, the animal walked stiff-legged and wandered restlessly. There was twitching and fasciculation of muscles but only after extremely high dosages were convulsions seen. Pulmonary rales developed shortly before death. Death occurred as a result of suffocation (Radeleff and Woodward 14; Smith and Jones 17; and Jolly 6).

Necropsy Findings

The post mortem lesions were very minor or were entirely absent in acute cases of poisoning. If lesions appeared, they were in the form of hemorrhages of varying size on the lungs, heart, and gastrointestinal tract. Lung congestion and edema was a prominent, but not necessarily constant, lesion. The trachea and bronchi often contained a frothy exudate. Pneumonia was observed if animals had been affected over a prolonged period

(Radeleff and Woodward 14; Smith and Jones 17; and Jolly 6).

Only minor histopathological changes were found in the tissues.

Treatment

Atropine blocked much of the parasympathomimetic activity of the organic phosphates if administered early in the course of poisoning and in twice the usual dosage. Animals should be maintained in a fully atropinized state for 24 hours or more (Jones 7).

Woodward (20) suggested the administration of atropine to cattle in two doses of 12.5 to 20 mg and 37.5 to 50 mg each. The atropine should be dissolved in 10 ml of distilled water, giving the smaller dose intravenously and the larger dose subcutaneously. This treatment was used in combination with 2-PAM (2-pyridine aldoxime methiodide) in a 50 mg/kg dose suspended in 100 to 125 ml of physiological saline and administered subcutaneously in the cervical region using 50 ml per injection site.

Because sheep required four to five times as much atropine as yearling cattle, the dosage should be adjusted accordingly. The amount varied in individuals, but in general, four to six mg/kg given in two or three doses was the most effective. Gordon (4) suggested suspending the atropine dosage in peanut oil for a more prolonged action.

Jones (7) stated that recovered patients may remain highly susceptible to organic phosphate poisoning for several weeks until the cholinesterase reserve may be replaced. This required about seven weeks in cattle.

MATERIALS AND METHODS

Six beef breed bulls, two to three years of age and purchased at a nearby stockyard were used in this experiment. There was no history available concerning general health or previous breeding records of these bulls. The bulls were ear tagged and placed in a box stall, two animals per stall. The ration consisted of alfalfa hay and a mixed grain concentrate in sufficient amount to maintain normal body weight and health.

Some difficulty was experienced in obtaining bulls that would respond to the electroejaculator. Therefore, three animals sprayed with Bayer 21/199 were compared to a control animal sprayed with water only. When two more animals were found that would respond to electroejaculation, one was sprayed with the experimental compound and one with water.

The bulls were closely confined in a portable chute. Preparation for collection included trimming the preputial hair, brushing litter from the lower abdominal wall, and wiping the area carefully with paper towels and cotton. Fecal material was removed from the rectum by a low water enema.

Semen samples were collected from each bull before and after spraying. The samples were obtained by use of the Marden ejaculator (William G. R. Marden, Fort Collins, Colorado) as pictured in Figure 1. Several attempts to collect samples by using the artificial vagina failed. Manton (10) reported no deleterious effects on fertility when using semen collected by the electrical method. Dzuick et al. (3) concluded that semen collected electrically was generally of greater volume but of lower density. This observation was also made in this study. The total number of spermatozoa were comparable to an ejaculate obtained by an artifical vagina. In this

Figure 1.

The equipment used for collecting samples is pictured in Figure 2. This consisted of a plastic centrifuge tube 33 by 100 mm fitted with a cork stopper, a conical 12 ml centrifuge tube which passed through a hole in the stopper, a latex collection sleeve and a holder equipped with a handle. The large plastic centrifuge tube was used to provide a 37°C water bath for the samples during collection and until examinations for motility could be completed.

After obtaining what appeared to be the most concentrated portion of the ejaculate the volume was recorded.

Motility estimations were made after gently mixing the sample. A large drop of semen was transferred to a clean glass slide warmed to approximately 37°C and examined under low power magnification. The proposed system of Herman and Swanson (5) for comparing degrees of motility was employed.

Figure 2.

The concentration of spermatozoa was determined by use of a Cenco type B2 photoelectric colorimeter (Central Scientific Co., Chicago, Illinois) as utilized by the Kansas Artifical Breeding Service Unit. This colorimeter had been standardized previously by hemocytometer counts and plotted logarithmically. One-tenth ml of semen was added to five ml of a three percent sodium citrate solution, mixed well and placed in the colorimeter for reading.

Slides for morphology examination of the semen were prepared within 10 minutes after collection. A small drop of semen was transferred to the surface of a clean glass slide and spread as thinly as possible by drawing a second glass slide across the surface. After air drying, the slides were stained for 10 minutes with a saturated solution of alcoholic eosin, washed gently with distilled water and allowed to air dry. Morphology examinations were made under oil immersion. One hundred sperm cells from various fields were counted and the percent of abnormal forms (Coffin, 2) recorded.

Semen for livability studies was diluted by adding 0.5 ml of fresh semen to 25 ml of extender solution which had been warmed to 37°C and stored in four ounce prescription bottles. The extender solution was prepared before each sample collection by mixing one part of pure egg yolk to three parts of three percent sodium citrate solution. The extended semen samples were placed in a 37°C water bath and gradually cooled in the refrigerator to approximately 8°C. These samples were examined under high power magnification at 24 hour intervals and results were recorded daily until the samples showed only 50 percent motility. Samples in this study were not examined after five days.

Equipment used in sample collection and storage was cleaned by washing in boiling tap water. The latex collection sleeve was soaked in 70 percent ethyl alcohol for 30 minutes, then rinsed several times in distilled water, and permitted to air dry. The glassware was rinsed several times in distilled water and dried in an oven.

Four animals used in this experiment were sprayed with a 0.5 percent solution of Bayer 21/199 (Chemagro Corp., Kansas City, Missouri). This solution was prepared by adding 12.5 ounces of 25 percent wettable powder to five gallons of water. The two control bulls were sprayed with water only. Five gallons of spray material was applied to each animal at 350 pounds pressure per square inch at each spraying.

Due to severe weather conditions, the temperature of each animal was recorded on the day of spraying and for seven days following.

One animal which had been sprayed with Bayer 21/199 solution was castrated unilaterally. Sections were taken from the testicle and fixed immediately in a 10 percent buffered formalin solution. After embedding, tissues were sectioned at six microns and stained with hematoxylin and eosin.

RESULTS AND DISCUSSION

In this study the bulls were divided, for experimental purposes, into two groups, four in one and two in the other. Both groups of animals were treated similarly, the only differences being the time of year that treatments were applied and the interval between spraying.

The results of the experimentation are given in the following tables. Tables 2 through 5 contain data derived from the four bulls of group one with regard to semen evaluation. Temperature studies on this same group

following each of two sprayings are given in Tables 8 and 9.

Studies on semen from the second group of bulls are presented in Tables 6 and 7. The results obtained on this group differed little from those on the first group. A discussion of the data is given following the tables.

Table 2. Semen evaluation of Bull 1 (Group 1) sprayed twice with Bayer 21/199.

Date	Total volume ml	Motility	Sperm per ml in millions	Total % abnormal	Livability in days
12/22	2.5	3	475	—	-
12/23	3.5	3	475	5	4
12/31	3	2	365	47	4
1/6	Sprayed with 5 gal. 0.5% Bayer 21/199				
1/9	4	3	775	5	5
1/20	4	2	653	14	-
1/23	2.5	2	400	14	4
1/26	1	2	455	25	3
1/28	Sprayed with 5 gal. 0.5% Bayer 21/199				
2/5	3.5	2	365	25	0
2/24	7	3	600	11	4
3/24	4	3	725	14	-
4/7	8	3	653	18	3
4/19	3	3	956	15	5

Table 3. Semen evaluation of Bull 2 (Group 1) sprayed twice with Bayer 21/199

Date	Total volume ml	Motility	Sperm per ml in millions	Total % abnormal	Livability in days
12/22	Sample mostly seminal fluids.				
12/23	2.5	4	1902	22	5
12/31	6.5	4	1902	48	5
1/6	Sprayed with 5 gal. 0.5% Bayer 21/199				
1/9	2.5	2	400	18	4
1/20	4	3	426	17	-
1/23	4	4	1801	12	4
1/26	2.5	4	1602	16	5
1/28	Sprayed with 5 gal. 0.5% Bayer 21/199				
2/5	3	3	825	12	4
2/24	4	2	400	23	3
3/24	5	2	365	8	-
4/7	-	-	--	--	-
4/19	4	2	268	18	4

Table 4. Semen evaluation of Bull 3 (Group 1) sprayed twice with Bayer 21/199

Date	Total volume ml	Motility	Sperm per ml in millions	Total % abnormal	Livability in days
12/22	Sample contained few sperm				
12/23	5	4	2003	14	5
12/31	5	4	2003	18	5
1/6	Sprayed with 5 gal. 0.5% Bayer 21/199				
1/9	3.5	4	1040	14	5
1/20	3	4	775	18	-
1/23	1	4	1159	16	5
1/26	2.5	4	1602	22	5
1/28	Sprayed with 5 gal. 0.5% Bayer 21/199				
2/5	4	4	1852	30	4
2/24	4	4	1040	--	4
3/24	6	4	1203	8	-
4/7	--	-	--	--	-
4/19	8	2	653	28	4

Table 5. Semen evaluation of Bull 4 (Control) Group 1 sprayed twice with water

Date	Total volume ml	Motility	Sperm per ml in millions	Total % abnormal	Livability in days
12/22	5.5	2	600	--	-
12/23	3	3	626	20	2
12/31	2.5	2	365	26	2
1/6	Sprayed with 5 gal. water				
1/9	4	3	825	20	3
1/20	2.5	3	400	14	-
1/23	1	4	1159	20	5
1/26	4	3	400	21	3
1/28	Sprayed with 5 gal. water				
2/5	2	3	725	17	4
2/24	4.5	3	475	21	4
3/24	6	3	475	2	-
4/7	--	-	--	--	-
4/19	5	3	825	16	5

Table 6. Semen evaluation of Bull 5 (Control) Group 2 sprayed twice with water

Date	Total volume ml	Motility	Sperm per ml in millions	Total % abnormal	Livability in days
1/12	2.5	2	400	22	3
1/20	3.5	2	475	18	-
1/23	3	2	426	18	4
1/26	2.5	3	725	39	3
2/13	4	4	1702	12	4
3/10	3.5	2	400	22	3
3/12	3	2	525	36	2
3/26	--	-	--	--	-
1/2	3.5	1	168	--	-
4/28	3	1	--	--	-
4/30	3	4	1203	10	5
5/4	--	-	--	--	-
5/5	Sprayed with 5 gal. water				
5/6	4	2	400	7	2
5/11	Few sperm cells				
5/18	Sprayed with 5 gal. water				
5/19	4.5	2	400	52	2
5/22	3.5	3	956	36	5
5/26	4	2	475	16	5
6/3	5	3	1152	10	4
6/10	--	-	--	--	-

Table 7. Semen evaluation of Bull 6 (Group 2) sprayed twice with Bayer 21/199

Date	Total volume ml	Motility	Sperm per ml in millions	Total % abnormal	Livability in days
1/12	2	3	455	6	3
1/20	Unable to obtain sample				
1/23	Unable to obtain sample				
1/26	4	3	600	18	4
2/13	3.5	3	725	31	4
3/10	3	3	653	52	5
3/12	6	3	775	--	5
3/26	7	3	639	14	5
4/2	6	2	168	22	4
4/28	3.5	3	400	18	4
4/30	4	3	875	15	5
5/4	5	3	653	19	4
5/5	Sprayed with 5 gal. 0.5% Bayer 21/199				
5/6	4.5	3+	725	15	5
5/18	Sprayed with 5 gal. 0.5% Bayer 21/199				
5/19	4	3	875	42	4
5/22	3	3	1152	26	5
5/26	6	3	925	18	5
6/3	6	3	1505	48	4
6/10	5	3	1100	18	5

Table 8. Temperature recordings on Group 1 bulls following the initial spraying.

Date	Bull No. 1 °F	Bull No. 2 °F	Bull No. 3 °F	Bull No. 4 °F
1/6	99.8	100.2	100.6	101
1/7	100.2	99.8	100.2	100.5
1/9	101	101.2	101.5	100.5
1/10	100.4	101	100.2	99.6
1/11	100.6	101.4	100.8	100.6
1/12	101.2	100.6	100.6	101
1/13	100.4	100.2	101	101.4

Table 9. Temperature recordings on Group 1 bulls following the second spraying.

Date	Bull No. 1 °F	Bull No. 2 °F	Bull No. 3 °F	Bull No. 4 °F
1/28	100.8	101	99.8	100.6
1/29	101.4	100.8	100.4	101
1/30	99.8	102	101.5	100.8
1/31	101	101.6	100.4	100.6
2/1	100.5	100	101	101.8
2/2	100.4	100.2	101.6	101.4
2/3	101	100.8	102.2	100.6

Examination of 48 semen samples indicated that spraying breeding bulls with Bayer 21/199 had no deleterious effects on the quality of semen. Twenty four samples collected from bulls sprayed with water showed only minor variations.

An attempt was made to collect the most concentrated portion of the ejaculate. The volume of the seventy four samples collected did not vary excessively during the entire experiment. Volume of samples varied from 1 ml to 8 ml with an average of four and one half ml per sample. Although it is generally recognized that volume may differ greatly in individual bulls and may have little bearing on fertility, volumes of ejaculates were compared. Herman and Swanson (5) found the average volume of semen in bulls to range from 2.5 ml to 5.5 ml. This may vary in individuals from day to day and from the method of collection. A portion of the sample from bulls number three and four was accidentally lost during collection on 1/23. Urine contamination was observed in the sample obtained from bull number one on 2/2. This altered the density of cells and accounted for the sperm being dead at the end of 24 hours.

Motility remained relatively constant during the experiment. Variation in rates of motility between intervals of collection appeared. It was observed after the second spraying with Bayer 21/199 that the motility value of sperm from bull number two declined with successive collections. Lagerlöf (8) stated that good motility of spermatozoa is necessary, but does not constitute any guarantee that spermatozoa are capable of fertilization. Some authors suggest a motility rating of three represents an average sample. In these studies, it appeared that a high motility rating at the time of collection was reflected in a higher concentration of

spermatozoa and a longer maintenance of motility during storage.

Some authors consider an average ejaculate to contain approximately 500 million spermatozoa per ml. Lagerlöf (8) considered bulls in which the concentration was below 250,000 per cu mm to be of poor fertility. As was previously suggested, the electrical method of collection may alter the density of semen samples. While some samples fell below the average of 500 million per ml in this experiment, there were no serious alterations after spraying except in bull number two which showed a decline in numbers after the second spraying with Bayer 21/199. A similar observation was made in control animal number five after the first spraying with water.

During this study relatively few or no small heads, undeveloped cells or immature cells with protoplasmic droplets were observed. Most of the abnormality that was seen occurred in the form of heads separated from the tails. It is thought that this cell damage occurred during slide preparation as it was not observed at the next collection. The slide preparation of bull three on 2/24 showed all cells to have been damaged by the preparation, as no abnormal forms could be observed in the extended sample.

Williams (19) stated that in ideally healthy sires the spermatozoa are strikingly uniform in morphology. Bulls of fairly high fertility may show 160 to 170 per 1,000 but beyond that ratio the evidence indicates low fertility with a high incidence of intra-uterine disease. Bulls in which 30 percent abnormal forms appeared did not reflect poor fertility. No one type of abnormality seemed to be more significant to infertility than another type (Herman and Swanson 5).

In the studies of motility of spermatozoa during storage, the semen of highest motility and greatest density remained most actively motile at

the end of the five day examination period. The greatest reduction of motility found in this study occurred during the first 24 hours of storage. On 1/20 there was a mechanical failure in the refrigeration equipment that froze and killed the extended samples from the four bulls.

Examination of samples for livability on 3/24 revealed all samples dead at the end of 24 hours. This was found to be due to a diluter solution that was highly alkaline. Urine contamination has a serious effect on spermatozoa as evidenced in a sample collected on 2/5 from bull number one. All spermatozoa in this stored sample were dead at the end of 24 hours.

There were no serious alterations in the storage quality of samples after spraying that could be detected.

There is general agreement that the maintenance of good motility in stored semen has a direct relationship to fertility. Pregnancies have been obtained from semen which has been stored up to 100 hours, although in artificial breeding units, samples are seldom utilized for insemmination beyond a three day storage period.

The four bulls used in Group 1 were examined for semen quality over a four month period and for approximately three months following the second spraying. This was considered a sufficient length of time for a complete cycle of spermatogenesis.

Due to the severe weather during the period of spraying Group 1, temperatures were recorded on the day of spraying and for seven days following. Lagerlöf (8) and Roberts (16) suggested that fever may cause disturbances in spermatogenesis. There appeared to be no thermal response as indicated in Tables 8 and 9.

Only minor changes in semen quality were observed in Group 2 after spraying bull number six with Bayer 21/199 and the control bull five with water. During the period 3/26 to 4/23, extremely poor samples were obtained from bull number five. The samples consisted mostly of epithelial cells with few sperm present. Bull number six samples remained fairly constant in motility, density, and livability. There were no significant changes observed on the spermatozoa during this study. Poor technique in slide preparation was suggested by the high percent of abnormal forms on 5/19 and on 6/3 in slides from bull six. Samples obtained from bull five were contaminated with urine on 5/4 and 6/10.

Further evidence that spermatogenesis was not severely disturbed as a result of spraying with Bayer 21/199 was obtained from the examination of the histopathology of the testicle removed from bull six. No pathological changes could be detected in the tissue sections.

Of the total of five gallons of spray material applied at each spraying, approximately three and one half gallons remained on each animal. Toxic symptoms were not observed after spraying. All animals continued to maintain good appetites, normal health, and ability to serve during the entire period of examination.

SUMMARY

Bayer 21/199, an organic phosphate compound, had no deleterious effects when tested on four beef breeding bulls in the form of a spray application of 0.5 percent in water solution. Evaluation was based on gross and microscopic examination of the semen and microscopic examination of one testis from one bull. Factors studied included the volume, motility, concentration, percent of abnormal forms, and livability. These factors

also included histopathology studies and ability to serve females in estrus.

Semen samples were examined during a fourteen week period which is a sufficient interval for a complete cycle of spermatogenesis.

The above evaluation should not preclude the possibility of semen quality being altered in the case of highly susceptible individuals which may develop toxic symptoms, even though treated with the recommended dosage.

An acceptable semen quality does not indicate that a male is always fertile but should only suggest that no changes have been noted which would indicate a reduced fertility.

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THE EFFECTS OF AN ORGANIC PHOSPHATE COMPOUND (BAYER 21/199) ON THE SEMEN QUALITY OF BULLS

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The most common cause of disturbances of spermatogenesis in males is due to degenerative changes in the epithelium of the seminiferous tubules. This degeneration may be the result of a multitude of causes such as hormonal disturbances, toxic influences, fever, or nutrition.

Some chemicals may cause sterility in animals but only two have been proven to do so. The semen quality of bulls is seriously affected for approximately 10 months following experimental poisoning with highly chlorinated napthalenes. Seminal degeneration will occur in rams after dipping in arsenic solutions. One worker suggested that organic phosphates have a lethal effect on the fetus.

Organic phosphates have gained widespread use in the livestock industry for the control of insects resistant to older insecticides. Poisonings in livestock following contact with organic phosphates have been reported. The toxic dosage varies widely due to individual susceptibility or variations in cholinesterase reserves.

Semen samples were collected and studied from six bulls. Four of them were sprayed with an organic phosphate compound, Bayer 21/199, and two bulls were sprayed with water only.

The results of this experiment indicate that the volume, motility, concentration, percent of abnormal forms of spermatozoa, and the duration of motility in stored samples was not affected by two spray applications of Bayer 21/199 when applied in the recommended 0.5 percent solution. This evaluation was based on samples studied before and after spraying with the compound. Semen samples were examined over a fourteen week period after spraying which is sufficient time for a complete cycle of spermatogenesis.

This study does not suggest that sterility would not develop in bulls that are highly susceptible to Bayer 21/199.