Table V. Interference of Various Materials in Determination of Dieldrin

Material	Apparent Dieldrin, Wt. %: Reduction–Phenyl Azide Method omitting chromatography; column A	with chromatography; column B	Chlorine method with chromatography; column C
Aldrin, 99.5%	1.5, 1.6	(<0.1)[a]	0.4
ASP-47, redistilled	0.0, 0.0	...	...
γ-Benzene hexachloride, recrystallized	0.1, 0.1	(<0.1)[a]	0.2
Chlordan, technical	2.8, 4.5	0.4, 0.5	4
DDD, recrystallized	...	...	<1
DDT	0.1, 0.1	(<0.1)[a]	<0.1
Endrin, 99%	0.1, 0.2	(<0.1)[a]	11
Heptachlor, recrystallized	0.2, 0.8	(<0.1)[a]	<1
Isodrin, 99%	...	...	<1
Methoxychlor, 90% technical	0.5, 0.6	(0.2)[a]	27
Octacide 264, technical	>10, >10	0.1, 0.1	...
Parathion, 96.5%	0.1, 0.1	...	...
Toxaphene, technical	9.3, 9.3[b]	1.6, 1.8	11

[a] Value estimated from extent of interference (column A) and extent of separation (column C).

[b] Interference presumably due to formation of double bonds during reduction, since Toxaphene gives only 0.1% interference by aldrin method (*5*).

Specificity of the Methods

A number of chlorine-containing materials were analyzed by the chlorine method (Table V). As halogen-free materials would not be determined by the method, they were not tested. While the method was shown to be somewhat specific, owing to the separations achieved by chromatography, four of the insecticides tested interfered from 4 to 27%.

Initial tests were made by the spectrophotometric method omitting chromatography. All but four of the materials tested interfered to less than 1% as apparent dieldrin (Table V). Further tests by the method including chromatography were made using three of the materials which interfered to more than 1%. The materials interfered from 0.1 to 1.8% in these tests. Comparison of the data in Table V shows that the spectrophotometric method was more specific than the chlorine method.

Acknowledgment

The authors wish to acknowledge the assistance of Bessie K. Chin, who performed many of the analyses reported in this paper.

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RODENTICIDES

Comparative Toxicities of Warfarin and Some 2-Acyl-1,3-indandiones in Rats

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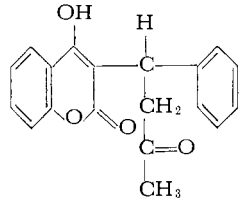
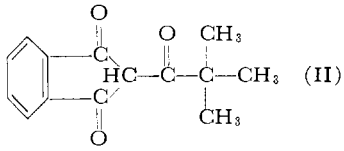
THE DEVELOPMENT and use of anticoagulant rodenticides within recent years has provided an efficient countermeasure to the ever-increasing rat population. The compound 3-(α-acetonylbenzyl)-4-hydroxycoumarin (I) was first described and tested by Link and his coworkers in 1944 (*7*). This material, now known as warfarin (after Wisconsin Alumni Research Foundation), is used widely as a rodenticide by military and civilian agencies (*3, 9*).



(I)

Warfarin is incorporated into a bait in low concentrations, and the bait is applied by the multiple-dose technique (*2*). Usually, an initial dilution in the form of a 0.5% powder with cornstarch is made. From this stock material, final dilutions of 0.025% warfarin are prepared with corn meal, rice meal, or other grain material. Because of the low concentration of warfarin in the final bait used, there is little danger of poisoning to other animals and man due to accidental single ingestion of the bait. Rats and mice feed for many days on the baits before becoming fatally poisoned. It is this "built-in" protective feature that makes the anticoagulant rodenticides so valuable.

In 1942, Kilgore (*5*) discovered that certain 2-acyl-1,3-indandiones were insecticidal in nature. Later, Kabat (*4*) described the anticoagulant properties of these compounds and also their acute toxicities. Of the compounds that he studied, the most effective material from the standpoint of anticoagulant and rodenticidal action was 2-pivalyl-1,3-indandione (II), commonly called Pival.



(II)

The water-soluble sodium salt of this compound, Pivalyn, is equally effective. Field tests of this material by the Branch of Predator and Rodent Control, Fish and Wildlife Service, soon disclosed that

One of the advantages of warfarin as a rodenticide lies in its low single-dose toxicity compared with its relatively high multiple-dose toxicity. 2-Pivalyl-1,3-indandione (Pival) is now being used as an anticoagulant rodenticide in the same manner as warfarin, and it was thought desirable to compare several other 2-acyl-1,3-indandiones with Pival and with warfarin for rodenticidal activity. Rats were injected intraperitoneally with the anticoagulants at various daily dose levels for 5 days. The most toxic compound tested was 2-diphenylacetyl-1,3-indandione, which produced a mortality of 39% at a dose of 0.1 mg. per kg. per day and 88% at 0.5 mg. per kg. per day. Warfarin, 2-phenylacetyl-1,3-indandione, and Pival were next in order of decreasing toxicity. The iron, copper, and sodium derivatives of the indandiones showed the same order of toxicity as the parent compounds. When tested for single-dose toxicity, warfarin was the least toxic, causing only 20% mortality in doses up to 100 mg. per kg. The indandiones ranked in toxicity as in the multiple-dose experiments, diphenylacetylindandione producing a mortality of 40% at a dose of 5 mg. per kg. Some protection was given to rats when vitamin K_1 was administered with daily doses of warfarin and diphenylacetylindandione.

Pival (and Pivalyn) in concentrations of 0.025% in cereal baits will effectively control infestations of Norway rats, roof rats, and house mice. In addition, the resulting cereal baits are less susceptible to insect and fungus contamination than baits prepared with warfarin (*2*).

The present report is concerned with a comparison of the toxicity of several new indandiones with that of warfarin and 2-pivalyl-1,3-indandione. Some additional observations were made on the effect of vitamin K_1 on the toxicity of these anticoagulant compounds. Most of the compounds used in this study were obtained from commercial sources; the 2-benzoyl-1,3-indandione and the copper and iron derivatives were prepared by the authors.

Procedure

Wistar male albino rats weighing 150 to 300 grams were used for this study. At least one group of six rats was used at each dose level of every compound tested. Where compounds compared closely with one another in toxicity or where results were questionable, several such groups were used. Compounds were administered intraperitoneally or orally. Where possible, the chemicals were dissolved in saline or in diethylene glycol monoethyl ether. Low concentrations of diphenylacetylindandione were prepared in mixtures of propylene glycol and saline. When the compounds were insoluble in an injectable solvent, suspensions in peanut oil were prepared. Animals were observed for 7 days, and mortalities noted. Necropsies were performed on animals that died without obvious external evidence of hemorrhagic death.

When the drugs were administered in daily doses, the same general procedure was employed. Injections were made each day for 5 days, the animals being observed for at least 7 days after the initial dose.

To test the effect of vitamin K_1 emulsion on the mortality produced by diphenylacetylindandione and warfarin, the vitamin K_1 was administered intraperitoneally at the same time as the daily dose of the drug. A few groups of rats receiving single doses of diphenylacetylindandione were given single doses of the vitamin K_1 at the same time.

Results

The single-dose mortalities of the more toxic compounds are given in Table I. Of the four compounds tested, diphenylacetylindandione is the most toxic in single doses. The other three compounds are listed in order of decreasing toxicity. Warfarin is least toxic of all in single doses. It produces approximately the same level of mortality whether given in doses of 25, 50, or 100 mg. per kg.

Table I. Mortality in Rats After Single Injections

Indandione	Dose (I.P.), Mg./Kg.	% Mortality	No. of Animals Used
Diphenylacetyl	1	0	12
	5	39	18
	10	39	18
	25	67	24
	50	83	18
Phenylacetyl	5	0	12
	10	25	12
	25	50	12
	50	92	12
Pivalyl	10	0	6
	25	17	6
	50	42	12
Warfarin[a]	1	0	12
	5	11	18
	10	13	24
	25	21	24
	50	17	18
	100	13	32

[a] Not an indandione.

The results of giving daily doses of the various compounds are shown in Table II, where the compounds are ranked in order of decreasing effect. Here again, the diphenylacetylindandione is the most potent compound. In this case, unlike that of the single-dose experiments, warfarin is almost as effective as diphenylacetylindandione. The last two compounds in Table II, benzoylindandione and diacetylisovalerylmethane, are ineffective even at a dose of 10 mg. per kg. per day.

Table III shows the effect on mortality of the daily administration of vitamin K_1 emulsion with diphenylacetylindandione and with warfarin. It is apparent that vitamin K_1 offers some protection against low daily doses of warfarin or diphenylacetylindandione. However, if the dose of either of these compounds is increased to 5.0 mg. per kg. per day, no further protection can be obtained with vitamin K_1, even though the daily dose of the latter compound is raised to 10.0 mg. per kg.

Discussion

Of the compounds considered here, four are outstanding in their ability to produce death in rats when given in small daily amounts. In order of descending potency these are 2-diphenylacetyl-1,3-indandione, warfarin, 2-phenylacetyl-1,3-indandione, and 2-pivalyl-1,3-indandione.

From the data, it seems probable that metallic derivatives of the indandiones such as iron, copper, and sodium are about as effective as the free acids in producing mortality. However, the physical properties of the heavy metal derivatives are very different from those of the free acids. Thus the iron and copper compounds of the indandiones, like the free acids, are very insoluble in water, whereas the sodium salts are somewhat more water-soluble. The free acids and

the sodium salts are bright yellow. The iron compounds are brick red in color and the copper compounds are a brilliant emerald green. Further studies of these metal derivatives are being conducted in this laboratory.

If a comparison is made between the effects of single doses and small daily doses (Tables I and II), a few points become apparent. Diphenylacetylindandione is the most potent compound, whether given in a single dose or in small daily doses. Warfarin, given in a single dose, is the least effective compound of the four considered, but given in small daily doses it becomes almost as effective as diphenylacetylindandione. This difference points to a possible environmental health problem in connection with the use of indandione rodenticides. One of the main safety features claimed for warfarin arises from the fact that a rodent must eat the bait for several successive days for death to occur (*8*). Since every 100 grams of bait contains only 25 mg. of rodenticide, it is unlikely that a domestic animal or human could consume a lethal amount of toxic material if the bait were eaten once by chance (Table I). Although the authors found some variation among the single-dose mortality experiments, it is clear that the potent indandiones are more toxic than warfarin. This finding suggests that the possibility of fatal accident with rodenticidal baits would be increased if indandiones were used.

The susceptibility of humans to death by warfarin poisoning is not much less than that of rats. This is made clear by the recent report of Lange and Terveer (*6*) in which a 19-year-old male Korean, previously in good health, ate sufficient corn meal containing warfarin to obtain an average daily ingestion of 50 mg. of warfarin (about 0.8 mg. per kg. per day). Death occurred on the fifteenth day after total ingestion of about 750 mg. of warfarin. In this case, the corn meal was eaten in the belief that the material was fit for human consumption. The possibilities that such an accident could occur in the United States may be small, but in countries suffering the ravages of war and invasion incidents of a similar nature could recur.

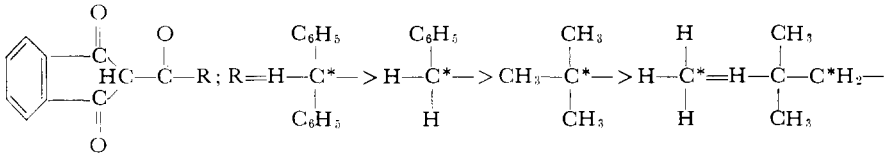
Kabat and others (*4*) reported that vitamin K in the form of 2-methyl-1,4-naphthoquinone had little if any effect against 2-isovaleryl-1,3-indandione in rats, while Beauregard and others (*1*) have recently reported vitamin K_1 (Mephyton) to be a more effective antidote to 2-pivalyl-1,3-indandione poisoning in dogs than synthetic vitamin K (Synkavite). The authors found vitamin K_1 (Mephyton) effective against low daily doses of diphenylacetylindandione and warfarin in rats. Animals receiving single doses of 50 mg. of diphenylacetylindandione per kg. and 5 mg. of vitamin K_1 emulsion per kg. experienced a mortality of 33% as compared with a mortality of 83% in rats receiving diphenylacetylindandione alone. From these findings it can be concluded that vitamin K_1 (Mephyton) would be a useful therapeutic agent in cases of accidental poisoning by the indandiones or warfarin.

Table II. Minimal Daily Dose of Rodenticide Producing at Least 50% Mortality in Rats in 7 Days[a]

Indandione	Daily Dose, Mg./Kg.	Total Dose, Mg./Kg.	% Mortality	No. of Animals Used
Diphenylacetyl	0.1	0.5	39[b]	18
Warfarin[c]	0.5	2.5	83	6
Diphenylacetyl Fe^{++} salt	1.0	5.0	100	18
Phenylacetyl	5.0	25.0	92	12
Pivalyl Fe^{++} salt	5.0	25.0	83	12
Pivalyl	5.0	25.0	67	12
Isovaleryl Fe^{++} salt	10.0	50.0	92	12
Isovaleryl	10.0	50.0	67	6
Isovaleryl Cu^{++} salt	10.0	50.0	50	6
Isovaleryl Cu^{+} salt	10.0	50.0	50	6
Acetyl	10.0	50.0	50	6
Benzoyl[d]	10.0	50.0	0	12
Diacetyl isovaleryl methane[c,d]	10.0	50.0	0	12

[a] Mortality data are for intraperitoneal injection only. A series of tests in which compounds were administered via the oral route gave similar results.
[b] In one group of 6 animals injected at this daily dose, no mortalities resulted. In two other groups of 6, 50 and 67% mortalities resulted.
[c] Not indandiones.
[d] Included for comparison.

The series of compounds studied here for their toxic action may be of interest in determining effect of chemical structure on physiological activity. If more toxic indandiones are ranked in order of decreasing activity, the following series of structural formulas is obtained:



The most potent structure contains the diphenylacetyl group. The branched-chain pivalyl compound is more toxic than its isomer isovalerylindandione. These facts suggest that the electronic influences on the carbon atom, marked with an asterisk, may be important in determining extent of over-all physiological activity of the compound. The presence or absence of metal elements appears to have no appreciable effect on the toxic action of the complete molecule.

Acknowledgment

The authors wish to express their appreciation to the following for their courtesy in supplying the experimental compounds: Motomco, Inc., for 2-pivalyl-, 2-isovaleryl-, 2-acetyl-, and 2-phenylacetyl-1,3-indandiones; Upjohn Co. for 2-diphenylacetyl-1,3-indandione (Dipaxin); Shell Development Co. for diacetylisovalerylmethane; and Merck & Co., Inc., for vitamin K_1 (Mephyton).

Table III. Effect of Vitamin K_1 Administered to Rats in Five Daily Doses

Rodenticide Dose (I.P.), Mg./Kg./Day	% Mortality Without Vit. K_1	Vitamin K_1 Dose (I.P.), Mg./Kg./Day	% Mortality with Vit. K_1
Effect on Diphenyl-acetyl-indandione Mortality)			
0.5	89 (18)[a]	2.0	0 (6)[a]
1.0	100 (18)	2.0	17 (6)
5.0		2.0	83 (6)
5.0	97 (34)	5.0	100 (10)
5.0		10.0	83 (12)
Effect on Warfarin Mortality			
1.0	88 (24)	5.0	33 (6)
1.0		10.0	17 (6)
5.0	94 (18)	5.0	100 (6)
5.0		10.0	67 (6)

[a] Figures in parentheses indicate number of animals used.

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COMPARISON OF PHOSPHATE FERTILIZERS

Fertilizer Value of Calcined and Fused Phosphates on Typical Soils

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A greenhouse experiment was performed in order to compare four —200-mesh, single-step thermal process phosphates with triple superphosphate and phosphate rock as sources of phosphorus for alfalfa and rye grass on ten typical soils of the United States. Rhenania phosphate, Coronet phosphate, and phosphate rock–magnesium silicate glass had nutritive values about equal to that of superphosphate when applied to calcareous soils of the western United States, while fused tricalcium phosphate had a lower nutritive value. Response to phosphorus application was low on these soils. The furnace phosphates and superphosphate gave comparable results with alfalfa on the midwestern, southeastern, and Mississippi blackbelt soils; with rye grass, Rhenania phosphate gave a lower nutritive response than the other materials. Phosphate rock showed little or no response over the no-phosphorus treatment on all the soils.

Phosphate fertilizers manufactured by single-step thermal processes have received considerable attention for many years. This class of products consists of water-insoluble citrate-soluble phosphates, which, being alkaline in reaction, cause evolution of ammonia when they are placed in contact with ammonium salts and moisture. As these materials are not well suited, therefore, for general use in mixed fertilizers, their place in the fertilizer economy must be found largely in cropping programs that call for application of phosphate either alone or in conjunction with potash, for example, as may be required in the growing of legumes and sod crops.

Among the single-step thermal treatments that have been developed on a commercial scale are: (I) sintering or fusion of phosphate rock with a suitable complement of silica in the presence of water vapor, whereby nearly all the fluorine in the rock is evolved, as in the cristobalite process (*25*) formerly utilized by the Coronet Phosphate Co. to produce a feed supplement, or in the fusion process (*10*) used by the Tennessee Valley Authority; (II) fusion of phosphate rock with magnesium silicate (*24*), as formerly done on the West Coast by the Permanente Metals Corp. (*4*) and Manganese Products, Inc. (*14*); and (III) calcination of phosphate rock with alkali salts—for example, with sodium carbonate as in the Rhenania process operated in Germany (*9*).

During the past few years considerable experimentation with production and use of Rhenania-type phosphate of the kind made in Germany has been conducted in the Rocky Mountain region. This product has shown favorable crop response on calcareous soils (*20*), even as good as the water-soluble phosphates, which are generally preferred for use on these soils (*2*). On the other hand, defluorinated phosphate rock has been reported to be relatively ineffective on calcareous soils (*5–7, 13*), as has also its principal constituent, α-tricalcium phosphate (*17*). The marked differences among observed crop responses to these phosphates suggested the need for an experiment that would permit a direct comparison of the several types of materials from single-step thermal processes on typical soils representing several of the great soil groups of the United States. Such an experiment was conducted in the greenhouse and results are presented here.

Table I. Composition of Phosphate Materials

Phosphorus Carrier	Sample No.	Mesh	P_2O_5, % Total	P_2O_5, % Available	P_2O_5, % Water-soluble	CaO, %	Na_2O (MgO), %	F, %
Triple superphosphate	3032	−28	50.0	48.8	44.0	22.5	...	1.6
Rhenania phosphate	3025	−200	25.8	20.1	0.3	37.4	16.6	2.9
Coronet phosphate	3022	−200	38.8	36.0	0.1	49.1	5.5	0.03
Fused tricalcium phosphate	3031	−200	28.0	23.4	0.1	39.8	...	0.3
Phosphate rock–magnesium silicate glass	2497	−200	20.4	16.5	0.1	32.4	(17.1)	1.7
Phosphate rock	2968	−200	33.7	3.4	0.03	48	...	3.7