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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

004439

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: IPRODIONE - 1-Year Dog Study Review

FROM: Alex Arce 5-3-85
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TO: H. Jacoby PM21
Registration Division (TS-767)

THRU: Clint Skinner, Ph.D. 5-6-85
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Theodore Farber, Ph.D. 5/8/85
Chief, Toxicology Branch (TS-769)

Compound: IPRODIONE - Tox Chem # .470A

Registration No. 359-684, Acc. No. 255951

Registrant: Rhone-Poulenc Inc.

Action Requested

To review the submitted data, 1-year Toxicity Study in Dogs.

1 of 3

004439

DATA EVALUATION REPORT

Chemical: IPRODIONE (Rovral)

Test Material: Iprodine Technical, 96.5%
1-isopropyl carbamoyl-3-(3,5-dichlorophenyl)-hydantoin
from Rhone-Poulenc Agrochimie S.A.

White Powder, Batch # DA 237-OF 81461

Study Type: 1 year chronic feeding study, dogs.

Study Identification: "52 week Toxicity Study in Dietary Administration to Beagle Dogs."
By Life Science Research Limited
Report No. 34 RR 0022/179
Sept. 28, 1984

Study Submitted to EPA by Rhone-Poulenc Inc.

MRID # Not assigned
Acc. # 255951

Purpose

To fill data gaps.

Methods : Attached copy from report, methods satisfy guidelines

Conclusion

Core Classification: Guideline
NOEL = 100 ppm
LEL = 600 ppm; Hematopoietic changes, the RBC, Hgb & Htc counts were lower than the controls.

The weight of the prostate gland was lower than the controls. Dose levels 0, 100, 600 and 3600 ppm.

At the high dose level the weights of the liver and the adrenal glands were higher than the controls. The weights of the prostate gland and the ovaries were lower than the controls. These changes were treatment related.

Histological changes in the liver and the urinary bladder were compound related.

The urinary bladder showed the most marked lesions, as granulomas and submucosal crystals.

Hematology and blood chemistry at the 600 and 3600 ppm Heinz bodies were observed, larger numbers at the 3600 ppm. The most

significant blood chemistry change was a higher value of alkaline phosphatase at the high dose level.

Discussion

The observed changes are indicative of toxic effects of the material at the higher dose level of 3600 ppm.

The target organs were the liver and the urinary bladder. The presence of Heinz bodies in the red cells is indicative of chemical insult.

Thus, the product is toxic at the high dose level, showed borderline toxic effects at the 600 ppm and no effect at the 100 ppm.

Reviewed by:

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Approved by:

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Protocol: .Materials and Methods

Compound: Iprodione 96.5% Batch # DA 237-OP 81 461

Test Subjects: Fifty pure-bred male and female beagle dogs.

Young adults 15 to 17 weeks of age with a body weight between 2.7 and 6.8 kg from Balbeggie Kennels, Fife, Scotland.

Dose levels: Control, 0 ppm; 100 ppm; 600 ppm and 3600 ppm.

Six males and six females assigned to each dose level group.

Protocol:

Male and female, healthy, young adult, beagle dogs were fed the compound (IPRODIONE) incorporated into the daily diet for 52 weeks.

Dose levels of 100, 600 and 3600 ppm and 0 control were used. Each dose level was offered to six male and 6 female animals. The method used followed the Series 83-1 of the Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, EPA, November, 1982. The dose levels were selected from a preliminary study, (preliminary study was not submitted with this report). However, the selection of dose levels is acceptable.

Diet

A moistened diet, 650 g, was offered daily 7 days a week with the compound incorporated into it by means of a Kenwood mixer, using 30% water to reduce the amount of dust.

The diets were prepared each week and analyzed for homogeneity and stability.

The treatment lasted for 364 days at which time the terminal sacrifice commenced.

Results : Attached copy from report, tables discussed in review

Mortality: Only one animal (female 600 ppm) was sacrificed at the 51st week due to continuous febrile convulsions, "this death was not attributed to treatment"; the explanation is acceptable - No other animals died during the study.

Signs of Toxicity: No signs of toxicity observed (None reported).

Food Consumption: Unremarkable, comparable to controls.

Body Weights: Unremarkable, comparable to controls.

Physical Examination: Unremarkable, comparable to controls.

Ophthalmology: The following signs were observed more frequently among the treated animals than the controls. An incidence of retinal hyperreflection, usually bilateral, slight, was observed in the males and females with greater incidence at the 600 and 3600 ppm level than the controls (Table 4, pg. 39). The meaning of this occurrence is not understood. It appears to be compound related however.

Hematology

Red blood cells: at the high dose level the PCV, Hgb and RCB count was lower than the controls. The plaletet count was higher than the controls at the 3600 ppm dose level. The attached tables, extracted from the submitted data, illustrate the changes (Table 5J).

These incidences were compound related although the difference in counts were not overwhelming.

Heinz bodies were observed at the high dose levels, male and female and the incidence was higher than the controls throughout the study at the high dose level. The production of Heinz bodies has been associated with oxidative processes due to drug treatment (Hematology, W. Williams et al., McGraw Hill, page 381, 3rd Ed.)

The following tables extracted from the submitted data showed the difference. Appendix 9K.

The thromboplastin time of females at the 3600 ppm dose was higher than controls, throughout the study. This effect was less severe in the males. This incidence was compound related.

Blood Chemistry

The reported effects were observed at the high dose level only and were as follows:

Plasma alkaline phosphatase: higher than controls at the 3600 ppm dose; statistically significant ($P < 0.05$). The effect progressively declined in the control dogs, while the high dose level dogs (M & F) showed an increase, excluding one male dog. This effect is indicative of liver malfunction.

Other observed differences between treated animals and control followed no trend or pattern and, therefore, are not significant.

Urine Analyses

No different than the control.

Organ Weights

Liver - adrenal. At the high dose level, male and female organ weight of the liver and adrenal were higher than controls and higher in relation to the absolute and relative body weights. Tables 9-A & B attached. These effects were compound and dose related.

Prostate weights were lower at the mid (600 ppm) and the high dose levels; Tables 9-A & B. These effects were dose compound and dose related.

Macroscopic observation

Enlarged adrenals in 2 females, swollen liver in one male.

Microscopic observations

The organs that showed treatment related changes were the adrenal glands and kidney (Table 10; selected values). Apparent increases were seen in: adrenal pallid zona fasiculata and zona glomerulosa in high dose males and females, kidney lipofuscinosis (proximal tubule) in high dose males and mid and high dose females; liver, hepatic cord atrophy in high dose males and females; urinary bladder submucosal granulomas and crystals.

These effects are compound and dose related and are indicative of the toxicity of the material.

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Iprodione Toxicology Review

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The material not included contains the following type of information:

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be stomatocytic forms displaying a progression of stomatocytic alterations from uniconcave discs to spherostomatocytes [54].

THE CODOCYTE

The codocyte, or target cell, is characterized by relative membrane excess due to either increased red cell surface area or decreased intracellular hemoglobin content. In patients with obstructive hepatic disease, an increased total membrane cholesterol content resulting from a decrease in lecithin cholesterol acetyl transferase (LCAT) activity with a significant increase in the cholesterol/phospholipid ratio [55] leads to an absolute increase in cell surface area. In iron-deficiency anemia and thalassemia, codocytes have relatively excess membrane because of the reduced quantity of intracellular hemoglobin.

THE ACANTHOCYTE [56]

Acanthocytes are generated from normal red blood cells under conditions that alter their membrane lipid content. The mechanism of acanthocyte formation is unknown. Once produced, the shape is irreversible. A markedly increased membrane cholesterol/lecithin ratio is common to acanthocytes from patients with hepatocellular liver disease and abetalipoproteinemia.

THE DISCOCYTE-DREPANOCYTE TRANSFORMATION

The sickle cell, or drepanocyte, displays a characteristic variation of form on stained blood films. Most commonly encountered is the fusiform cell in the shape of a crescent with two pointed extremities. Such cells persist in well oxygenated preparations of sickle cell blood and have been referred to as *irreversibly sickled cells* [57]. In addition to these bipolar drepanocytes, examination of deoxygenated sickle cell preparations in the phase-contrast microscope reveals varied cell forms characterized by pointed extremities in various holly-leaf and poikilocytic configurations, many containing multiple spicules several microns in length. The spicules are quite fragile and easily evulse from the cell. As deoxygenation proceeds, the cell loses its flicker before shape change is evident [58]. This is followed by slight deformations at the border of the discocyte, with displacement of the hemoglobin to one region of the cell. After a few minutes of deoxygenation, characteristic spicules appear and the cell elongates and becomes rigid. Areas within the spicules and the cell center manifest varying degrees of birefringence, indicating an organization of hemoglobin molecules within the cell [59]. The hemoglobin S polymers are rods of 150 to 180 Å in diameter, which appear to be composed of monomolecular filaments of 60 to 70 Å in diameter intertwined into a six-stranded helix [60]. In partially sickled cells such polymers display random orientation, but as cells become more tightly sickled, polymeric rods of hemoglobin S undergo lateral alignment into paracrystals. The polymers of intracellular hemoglobin are long parallel rods aligned with the long axis of the cell or spicule. Upon reoxygenation, the sickled drepanocyte reverts to the discocyte form and in so doing loses membrane as the retraction of long spicules occurs, accompanied by the process of microspherulation and fragmentation [61]. The unsickling process also leads to the formation of micro-Heinz bodies that adhere to the internal surface of the red cell membrane and contribute to the increased membrane rigidity and cation leak [62]. With repetitive sickle-unsickle cycles, membrane damage accumulates until the cells become incapable of reversion to the biconcave disc shape even in the presence of high oxygen tension and in the absence of internal polymers. They thus become irreversibly sickled cells. The irreversibly sickled cell has a high hemoglobin concentration, increased cation permeability with decreased potassium and increased sodium content, and markedly decreased cellular deformability [57].

SHAPE ALTERATION IN HEINZ BODY–CONTAINING CELLS

Heinz bodies are particles of denatured proteins, primarily hemoglobin, which form as a consequence of chemical insult (Chap. 65), from hereditary defects of the hexosemonophosphate shunt (Chap. 58), or in the thalassemias (Chap. 50) or unstable hemoglobin syndromes (Chap. 61). The binding of denatured hemoglobin to the membrane leads to altered cation permeability with loss of water and potassium, sodium gain, and early ATP depletion [63]. Portions of the membrane damaged by Heinz body fixation may be lost from the cell by microspherulation or fragmentation, leading to spherocyte formation [64]. Heinz bodies appear as small rounded or angular inclusions measuring from 0.3 to 2 μm in diameter by light microscopy. They are easily seen in the phase-contrast or the interference microscope and strongly absorb Soret band (414 nm) light. Vital staining with crystal violet, new methylene blue, or brilliant cresyl blue easily demonstrates these inclusions. They persist after hemolysis and usually appear to be attached to the cell membrane. Heinz bodies are seen in films stained with May-Grunwald-Giemsa but are invisible in Wright's-stained films. In the electron microscope they appear as dense masses which begin to form in the center of the cell and then become attached to the red cell membrane [65]. Freeze-etch studies show Heinz bodies to occur as dense submembrane hemoglobin aggregates affixed to the internal membrane surface or as isolated large masses of denatured hemoglobin producing marked distortion of the overlying membrane [64]. The attachment of the Heinz body to the membrane causes a rearrangement of membrane-associated particles, with aggregation of these particles over the Heinz body regions, suggesting that denatured hemoglobin may be attached to membrane glycophorin and other proteins.

Electron microscopic studies have confirmed Heinz's original observations [66] of the inability of rigid Heinz bodies to traverse the interepithelial slits of the splenic sinus. These inclusion bodies are left behind in the perisinusoidal red pulp for phagocytosis by macrophages [67].

osmotic fragility which persists after papain treatment releases them from the monocytes. It is assumed that the monocyte partially phagocytoses or digests a portion of the red cell membrane, thereby producing a cell with a decreased surface-area/volume ratio; however, little is known about this process. Attachment to monocytes occurs whether IgG is bound to the red cell immunologically or by nonimmunologic means, such as chronic chloride or cephalothin. This process of attachment is inhibited by corticosteroids [87].

The spleen is particularly efficient in trapping red cells which are coated with IgG antibodies, but these cells are ordinarily not detected by the liver. However, with large amounts of antibody, the liver shares in this process [16]. When the number of antibody-coated red cells is small, they are completely cleared from the circulation by the spleen. No spherocytosis is observed in the peripheral blood, although spherocytes can be demonstrated in the spleen [88]. When large numbers of red cells are coated with IgG, there appears to be a backup into the circulation of red cells made spherical and then released [89]. Such spherical red cells are in double jeopardy as they recirculate through the spleen: they risk being bound again by phagocytes, and they are hindered in their passage through the splenic circulation because of their spherocytic shape.

Attachment of C3 Antibodies of the IgM class are capable of agglutinating red cells. In human disease these antibodies have a thermal amplitude with greatest activity at 4°C and usually no significant activity above 32°C, although in some patients activity extends to body temperatures [90]. The monocyte-macrophage system does not appear to have receptors which recognize IgM [84]. Rather, the mode of destruction of red cells in cold agglutinin disease appears to relate to the ability of IgM antibodies to fix complement. Since complement is fixed at warm temperatures, whereas IgM antibodies dissociate from the cell at these temperatures, this is not an efficient process, and fixation of sufficient molecules of C1 to permit ultimate cell lysis occurs only very rarely. Rather, cells become coated with C3 and, to a smaller extent, C4. Monocytes [91] and liver Kupffer cells [92] have receptors for C3 in its biologically active form (C3b). Degradation of C3b on the red cell surface to a form which is immunologically recognizable but biologically inactive (C3d) results in the circulation of C3d-coated red cells which are not in jeopardy [93,94] and which are actually more resistant than normal to the effects of IgM antibodies [93]. Human monocytes bind and sphere human red cells coated with C3b in a fashion totally analogous to that observed with IgG, and the process is inhibited by corticosteroids [95]. However, spherocytosis in the peripheral blood is uncommon, possibly because Kupffer cells, which appear to predominate in the clearance of C3b-coated cells, do not release partially injured red cells as readily as splenic macrophages. Coating of red cells with C3 is not a unique property of IgM antibodies, but may also occur when a high concentration of IgG antibodies is present on the cell surface. In both conditions there is a general correlation between amount of C3 present on the cell surface and the cell survival [96].

Spherocytosis appears to be a constant feature of red cells brought into close contiguity with monocytes or macrophages. Thus it occurs when red cells are bound to monocytes immunologically by means of IgG or C3b. It also occurs when red cells are bound to monocytes by the plant lectin concanavalin A in vitro [97] or when concanavalin A is injected into animals in vivo [98]. It is generally thought that the spherocytosis caused by splenic pooling of red cells results from the adverse metabolic conditions which exist within spleen cords engorged with red cells. Such engorgement may also provide the necessary degree and duration of contiguity between red cell and macrophage to permit this same process to occur in the absence of bridging molecules such as IgG, C3b, or concanavalin A.

Oxidation of membrane proteins Interference with the functional capability of membrane sulfhydryl groups, either by their oxidation or by their blockade with sulfhydryl-reactive reagents, e.g., *N*-ethylmaleimide (NEM) or parahydroxymercuribenzoate (PMB), results in premature red cell destruction in vivo [14]. High concentrations of sulfhydryl-active agents in vitro interfere with membrane permeability to cations and lead ultimately to osmotic swelling and hemolysis [55]. With mild sulfhydryl group injury, transfused red cells are destroyed exclusively in the spleen. However, with greater damage, destruction occurs both in the spleen and in the liver [14]. In order to achieve hemolysis in vitro the red cell injury must be considerable, while destruction of red cells in vivo may be observed using amounts of sulfhydryl inhibitors lower than those capable of causing changes in vitro. Thus the spleen filter is able to perceive very subtle membrane injury, the precise nature of which is unknown. Once trapped in the spleen, sulfhydryl-inhibited red cells undergo a progressive increase in osmotic fragility [14].

Most oxidant drugs are substituted benzene derivatives which act as free-radical intermediates facilitating reactions between molecular oxygen and proteins within the red cell. In the presence of oxygen these compounds stimulate generation of highly reactive free-radical forms of oxygen (superoxide, peroxide, hydroxyl free radical, and singlet oxygen) [99]. While hemoglobin is the protein most frequently oxidized (resulting in Heinz body formation), membrane sulfhydryl groups are also oxidized, and those drugs which are lipophilic have the greatest tendency to cause membrane rather than globin oxidation [100]. The result is spherocytosis and hemolysis [44]. Although it had been suggested that Heinz bodies themselves could block membrane sulfhydryl groups by forming mixed disulfides [101], no evidence of such disulfide formation has been found [102]. Nonetheless, Heinz body–containing cells, as in hemoglobin Köln [101], and α-chain–containing cells in β

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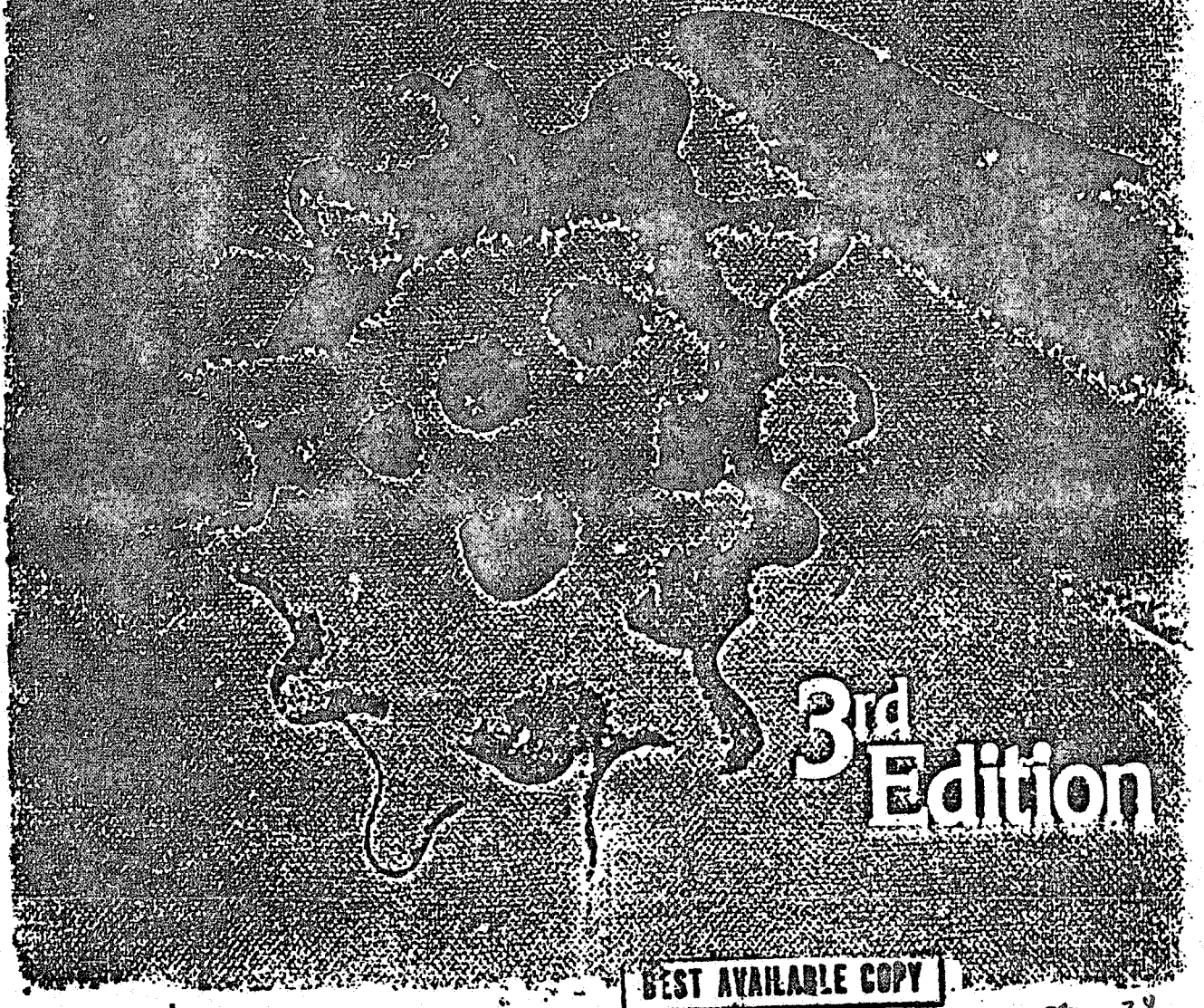

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