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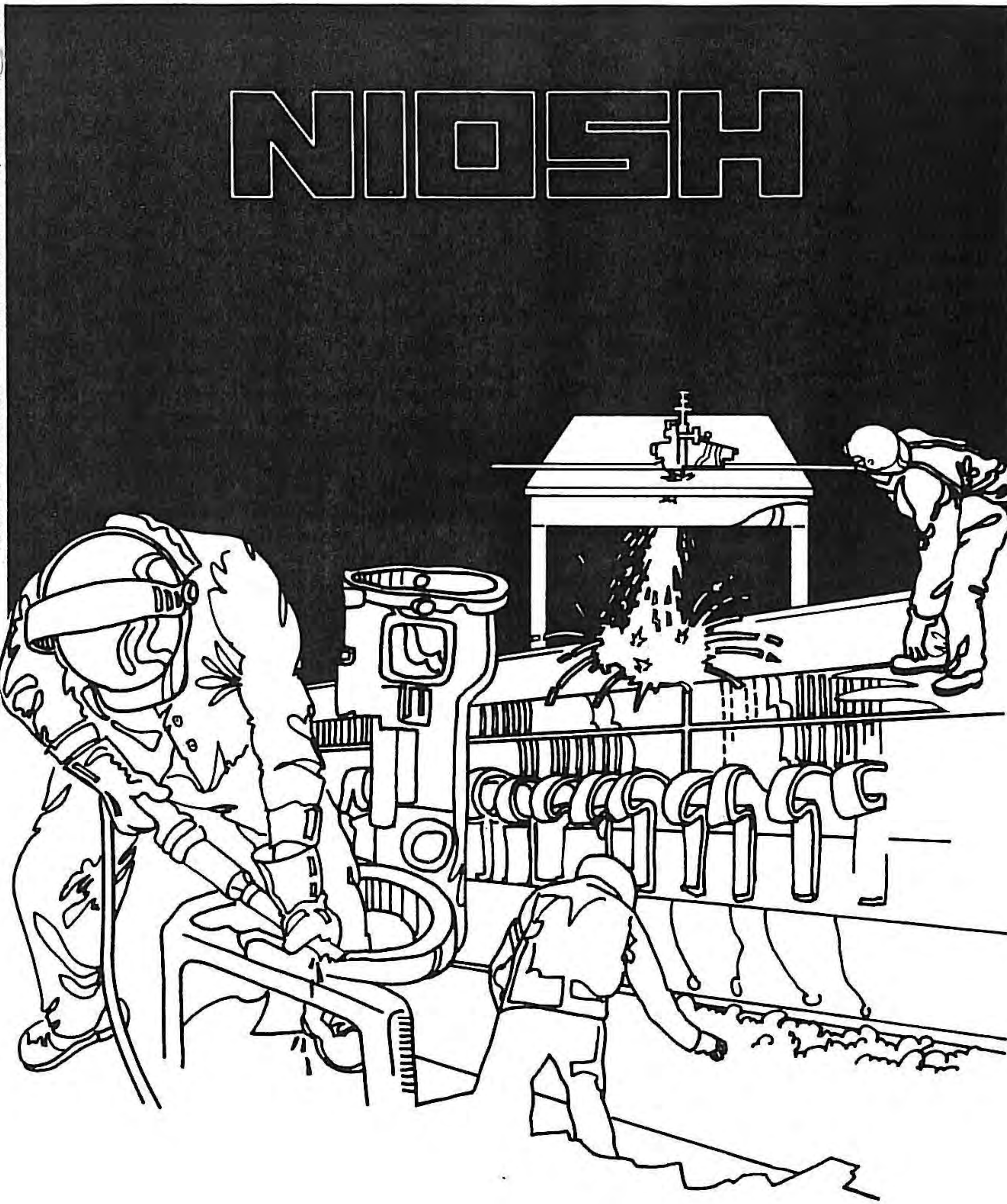

Health Hazard Evaluation Report

HHE 80-163-1026
PACIFIC GAS AND ELECTRIC/
WESTERN CANAL COMPANY
NELSON, CALIFORNIA

PREFACE

The Hazard Evaluations and Technical Assistance Branch of NIOSH conducts field investigations of possible health hazards in the workplace. These investigations are conducted under the authority of Section 20(a)(6) of the Occupational Safety and Health Act of 1970, 29 U.S.C. 669(a)(6) which authorizes the Secretary of Health and Human Services, following a written request from any employer or authorized representative of employees, to determine whether any substance normally found in the place of employment has potentially toxic effects in such concentrations as used or found.

The Hazard Evaluations and Technical Assistance Branch also provides, upon request, medical, nursing, and industrial hygiene technical and consultative assistance (TA) to Federal, state, and local agencies; labor; industry and other groups or individuals to control occupational health hazards and to prevent related trauma and disease.

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Pacific Gas and Electric/Western Canal Company
Nelson, California

NIOSH INVESTIGATORS:
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I. SUMMARY

On May 27, 1980, the National Institute for Occupational Safety and Health (NIOSH) received a request for Health Hazard Evaluation from Local 1245, International Brotherhood of Electrical Workers (IBEW), Walnut Creek, California. The potential hazard of concern was the exposure of canal maintenance workers to the ricefield herbicide Ordram.

Ordram is a thiocarbamate herbicide used to control aquatic grasses. In the laboratory, the herbicide has been shown to cause a reversible infertility in male mice and rats at doses of 1/1000 of the approximate oral LD_{50} of 655 mg/kg.

Canal maintenance workers are responsible for the general upkeep of the canals and adjacent roadways, and for metering and recording the volumes of water dispensed into the paddies from the main canal. The workers are subjected to inhalation and skin contact with the herbicide during and after its applications to the paddies.

A preliminary environmental study was conducted at Western Canal Company during June 16 to 18, 1980. The area was toured and observations were made of Ordram application methods and the work practices of employees. High-volume atmospheric sampling was performed for Ordram. The samples were not analyzed because an analytical method was not available at the time.

A follow-up environmental study was performed May 14 to 17, 1981. Air samples were taken for Ordram, caprolactam, hexamethyleneimine, and total mercaptans. A trace of Ordram (8 ppb) was detected on one area sample, but the remaining samples had non-detectable results.

An epidemiologic questionnaire administered to discern any reproductive difficulties among the 10 exposed workers gave no indications of reproductive problems related to their work.

A literature review of the existing studies regarding mammalian responses to Ordram is contained in the Appendix of this report.

NIOSH concludes that although the potential for reproductive interference by Ordram herbicide exists, the exposures encountered in the Western Canal situation are probably not of sufficient magnitude or duration to have discernable effects using presently available diagnostic techniques. Recommendations to help ensure a safe working environment are found in Section VII of this report.

KEYWORDS: SIC 0710 (Agricultural Services), herbicide, Ordram, caprolactam, hexamethyleneimine, mercaptans, infertility, reproductive hazard

II. INTRODUCTION

A request for health hazard evaluation was received May 27, 1980, from an authorized representative of the International Brotherhood of Electrical Workers (IBEW), Local 1245, Walnut Creek, California. The hazard suspected to exist was the exposure of canal maintenance men to the herbicide Ordram[R] (Stauffer Chemical Company, Westport, Ct.).

An opening conference was held at Pacific Gas and Electric Company (PG&E) corporate headquarters in San Francisco, California, on June 16, 1980. Present at the meeting were representatives of IBEW, PG&E, Stauffer Chemical Company, California Departments of Food and Agriculture, Health Services, and Industrial Relations, and the National Institute for Occupational Safety and Health (NIOSH). A preliminary environmental survey was performed during June 16-18, 1980 which included: observations on Ordram application methods, work practices of the employees in question, and high-volume air sampling for Ordram. A follow-up environmental survey was performed May 14-18, 1981, during the next application season. Included in the second survey was air sampling for Ordram, and three materials evolved through the processes of its environmental degradation: caprolactam, hexamethyleneimine, and ethyl mercaptan.

III. BACKGROUND

Western Canal Company is a subsidiary of Pacific Gas and Electric Company. The company operates and maintains a network of irrigation canals in Butte County, California, that provide water for about 65 square miles of rice paddies. Nelson, California, is situated west of Oroville in the Sacramento Valley or north central section of the state. The winters are moderate with considerable rainfall while the summers are characteristically hot and dry.

Short-grain rice culture in California is known for its lack of labor-intensive efforts to produce a crop. Fields are graded into checks using laser-directed automatic levelers. Older style fields which were leveled by surveyor transit and stadia rod methods follow the natural contures of the land, with the intent to have an elevation drop of between 2/10th and 3/10th inch per field or 'check', since rice requires constantly flowing water. Seed and herbicides are sown by airplane. The fields are flooded with water to an average depth between two and four inches. Weed control is achieved by regulating the depth of the field water at critical times and by the regular application of herbicides such as MCPA ([(4-chloro-*o*-tolyl)oxy]acetic acid) and Ordram.

The rice season in northern California runs from preparation and flooding in April through harvest in September. It is the primary duty of the canal maintenance men during this time to regulate the level of water in the rice checks by adjusting the flow gates which allow canal water into the paddies, and to record the volumes of water which are dispensed into the fields. During the time that water is not in the canal, they maintain the canals and roadways. Contact with water in the paddy and inhalation of ambient air are the two postulated routes of exposure. In California short-grain rice culture, Ordram is utilized in two formulations, often concurrently.

An emulsifiable liquid concentrate 8-E, that is 8 lbs active ingredient per gallon (.96 kg/l) is commonly metered into a paddy during the first flood at a rate of 3 lbs/a (3 kg/ha). It may also be incorporated into soil in good tilth, but immediately to prevent loss due to volatilization.

Another and sometimes more common method of application in similar situations is an air application of a granular formulation 10G, that is 10 lbs active per 100 lbs of product. Inert carriers such as clays make up the balance of this mix. Usually the liquid or "drip" application is followed by an air application. The two applications per field is common practice and in some circumstances, a third or "spot" treatment is performed. The rate for air application is similar to drip.

IV. EVALUATION DESIGN AND METHODS

Environmental

Personal and area sampling was conducted to determine values for exposure to Ordram, and three compounds associated with its environmental degradation. These compounds, hexamethyleneimine, caprolactam, and ethyl mercaptan were identified through the literature on Ordram.

Sampling for Ordram and caprolactam was performed using three types of mechanical pumps. A Gast high-volume pump equipped with a 14.1 or 7.5 l/min critical orifice was powered by a portable electric generator. Two personal size pumps, DuPont P-4000, and P-200 were also used. This gave a 100 fold sampling range since the optimum sampling rate and volume for the field detection of the herbicide was not known. Both materials were collected on two-section, large XAD-2 porous polymer tubes (SKC #226-30-02).

The above mentioned samples were desorbed with 2 ml methylene chloride containing 1 ul/ml of tetradecane as an internal standard and analyzed by gas chromatography according to NIOSH Method No. P&CAM 127 (modified) using a Hewlett-Packard 5731A gas chromatograph with flame ionization detector. A 6'X 1/8" stainless steel column packed with 20% SP-2100 and 0.1% Carbowax 1500 on 100/120 Supelcoport was used with temperature programming from 185 to 230°C at a rate of 4°C/min. The limit of detection was 10ug per sample for Ordram and 40ug per sample for caprolactam.

Hexamethyleneimine was sampled by using three section silica gel tubes (SKC #22-14) and analyzed by NIOSH P&CAM Method No. 221 (modified). The A, B, and C sections of the samples were separately desorbed with 0.4N HCl in 80% methanol. One-half of each acidic sample solution was then made basic with 1/2 ml of 0.5N NaOH in 80% methanol containing toluene as an internal standard. The sample solutions were subsequently analyzed using a Hewlett-Packard 5710A gas chromatograph equipped with a flame ionization detector. A glass column 3' X 1/4" long containing 80/100 mesh Chromosorb 103 was used. Oven temperature was 190°C. The limit of detection was 50ug per sample.

Special sampling tubes were prepared for ethyl mercaptan. They consisted of mercuric acetate coated glass wool inside of thin wall glass tubing. A colormetric reaction was induced and the results read on a spectrophotometer. The lower limit of detection was 2.5 ug per sample.

Epidemiological

The initial medical evaluation consisted of a questionnaire completed by the men working at each of three sites where exposures to Ordram might occur. After completing the questionnaires, the men from Western Canal sent them to NIOSH. All questionnaires were then forwarded to Dr. Whorton at Environmental Health Associates in Berkeley, California.

V. EVALUATION CRITERIA

No standard has been promulgated by any Federal regulatory or advisory agency. Stauffer Chemical has established 0.1 mg/M^3 as the threshold limit value for its workers exposed to the herbicide during its manufacture. This safe exposure level was estimated by extrapolation from no-effect levels observed in animal testing.

VI. RESULTS AND DISCUSSION

Environmental

Eight hour personal samples did not detect any exposures for any canal maintenance man to any of the suspected hazards. One high-volume area sample (7.5 l/min for 1 hour) reported a trace of Ordram (0.06 mg/M^3 or 8 ppb). Area samples taken at a metering station (#663) showed non-detectible levels for all suspected hazards. At the same location, area samples taken five feet immediately downwind from a 55 gallon barrel of Ordram which was being metered into the paddy showed all non-detecteds, except for the trace. This 342 acre paddy previously had at least 300 additional gallons introduced prior to the air sampling.

Proximity to a source of Ordram, therefore, does not necessarily constitute a hazardous exposure situation. Even though it may be perceived that appreciable quantities of the material have been introduced into the immediate vicinity of the metering stations, the general dilution ventilation afforded by winds are effective in dispersing any potentially consequential exposures. Additionally, the various field formulations of the herbicide are designed so that the solubility of the Ordram mixture is favored to be retained in the water matrix.

The odor of technical Ordram is clearly indicative of its general presence in the immediate vicinity. The production impurity, diethyl disulfide, is contained in the technical Ordram mixture at 1 to 2% by weight. Diethyl disulfide has an odor characteristic of 'rotton cabbage' and has a reported odor detection threshold of about 5 parts per billion (ppb). Reduction of diethyl disulfide yields ethyl mercaptan ('skunk-like odor') which can be detected by the human nose at levels between 0.02 and 0.4 ppb.

A serendipitous skunk kill provided a field source of butyl mercaptan (odor detection threshold about 1 ppb). The odor was subjectively judged to be intense. One set of samples was run for 20 minutes and the second set for about 1 hour to give a sample volume of 4 to 12 liters. All results were in the non-detectable range. Although the odor of Ordram is evident, the actual amounts of the impurity in the air are quite low.

In order to fully characterize a low-level occupational exposure, it would be desirable to measure very minute quantities of the suspected contaminant in the air. Although this was not possible during the study, the purposes of the environmental survey were fulfilled; that is, to determine an exposure range. In all cases, personal exposures to the suspected hazards were exteremely low, and therefore judged to be of a non-hazardous nature.

Epidemiological

At Western Canal, 11 questionnaires were distributed to current employees; two additional ones were mailed to former employees. Of those distributed, 10 were returned for a 77% return rate.

At Modesto and Merced, 12 were handed out (6 at each location); ten more were given to the business agent to distribute to the men not present at the time of the initial visit. Eleven of the 22 were returned (50%), eight from Modesto and three from Merced.

In total, of the 35 questionnaires distributed, 21 were returned for a 60% response rate.

Tables I, II, and III show the summary for all three groups. The mean age was 34.7 years for the men and 34.2 for their wives. They had been married for an average of 12.2 years, and had worked at their current jobs for an average of 6.6 years. The mean number of children for the couples wanting children was 2.3. Of the seventeen married men, 15 had fathered children. Eight had children born since employment in one of the three job locations under investigation. Not all of the married men had tried to father children.

The data obtained do not indicate any reported difficulty with fertility among men who responded to the questionnaire. Likewise, there were no excess miscarriages or stillbirths as compared to expected rates.

Conclusions

In a mixed genetic population, individuals will respond to a uniform exposure of a given agent in varying manners and degrees. That is to say, in this study, it is virtually impossible to separate an adverse health effect associated with exposure to Ordram from other agents the worker may be exposed to in his habits, diet, water, environment, or workplace. In this case, unless the workplace contaminant was an extremely potent agent, was present in substantial concentration, or produced particularly graphic symptoms, it could not be irrefutably said to cause the suspected effects.

The state of the art capabilities in evaluating a chronic, intermittant, very low dose exposure situation has not yet progressed to the stage at which definitive judgements can be made from a single field study. A small cohort, such as the group at Western Canal Company, does not provide a population of suitable size to utilize epidemiologic tools that have successfully provided insight into similar environmental/occupational exposure situations.

Since the respondents experienced no fertility problems or unusual pregnancy outcomes, and the exposure to Ordram is exceedingly low, there appears to be no indication to conduct further studies of testicular function, such as semen analyses. Unless new information becomes available to indicate a problem among these men, no further study is recommended.

VII. RECOMMENDATIONS

Canal maintenance men are encouraged to continue good work practices.

Workers at Western Canal should report any improperly placed or undisposed of Ordram^R drums.

VIII. AUTHORSHIP AND ACKNOWLEDGEMENTS

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X. DISTRIBUTION AND AVAILABILITY OF REPORT

Copies of this report are currently available upon request from NIOSH, Division of Standards Development and Technology Transfer, 4676 Columbia Parkway, Cincinnati, Ohio 45226. After 90 days, the report will be available through the National Technical Information Service (NTIS), 5285 Port Royal, Springfield, Virginia 22161. Information regarding its availability through NTIS can be obtained from NIOSH Publications Office at the Cincinnati address. Copies of this report have been sent to:

1. Authorized Representitive, Local 1245, International Brotherhood of Electrical Workers, P.O. Box 4790, Walnut Creek, California 94596.
2. Pacific Gas & Electric Company, 215 Market Street, San Francisco, California
3. Union Steward, Western Canal Company, Colgate Division, Nelson, California
4. U.S. Department of Labor/OSHA, Region IX
5. NIOSH, Region IX

For the purpose of informing affected employees, copies of this report shall be posted by the employer in a prominent place accessible to the employees for a period of 30 calendar days.

TABLE I

Mean Demographic Data on 21 Men Working in Areas with Potential Ordram Exposure

Age of men	34.7
Age of spouse	34.2
Years married	12.2
Years at current job	6.6
Number of children for men wanting children (N=14)	2.3*

* excludes one in utero

TABLE II

Results from 21 Men Working in Areas with Potential Ordram Exposure

Status or outcome	Yes	No	NA
Married	17	4	-
Fathered children	15	6	-
Youngest child born since current employment	8	7	-
History of difficulty with conception	1	14	6
Number of miscarriages (spontaneous abortions)	2	19	-
Number of stillbirths	0	21	-
Current cigarette smoker	7	14	-

TABLE III

Results of 21 Men by Specific Work Location
with Potential Ordram Exposure

	Western Canal N=10		Modesto Irrgtn Dist N=3		Merced Irrgtn Dist N=8	
	Yes	No	Yes	No	Yes	No
Married	8	2	3	0	6	2
Fathered children	7	3	3	0	5	3
Youngest child born since current employment	3	4	3	0	2	3
History of difficulty with conception (for men trying to father children)	0	7	0	3	1	4
Number of miscarriages (spontaneous abortions)	1	9	0	3	1	7
Current cigarette smoker	0	10	1	2	4	4

APPENDIX

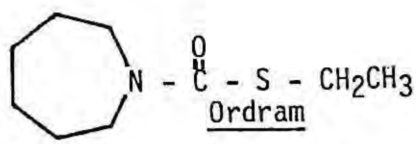
ORDRAM CHEMISTRY

The basic carbamic acid molecule $NH_2-\overset{O}{\overset{\|}{C}}-OH$ may have sulfur substituted for an oxygen to form thiocarbamic acid

$$NH_2-\overset{O}{\overset{\|}{C}}-SH.$$

Futher substitution is possible on the nitrogen and sulfur atoms

$$\begin{matrix} R_1 \\ R_2 \end{matrix} N-\overset{O}{\overset{\|}{C}}-S-R_3.$$



Ordram

Ordram is the common name for the technical mixture of the active ingredient, molinate (s-ethyl-hexahydro-1H-azepine-carbothioate). Ordram is mentioned in the literature by several other names, among the more common are: molinate, hydram, and yalan (Soviet). The mixture is approximately 97% parent compound by weight, with the production impurity diethyl disulfide accounting for 1-2%. The ratios vary from batch to batch.

Stauffer introduced the chemical in 1954. It is synthesized by reacting S-ethyl chlorothioformate and hexamethyleneimine. It is non-corrosive and fairly stable to hydrolysis[3,4].

At room temperature (20°C) Ordram is an amber liquid with a specific gravity of 1.063 and a boiling point of 137°C at 10 mm Hg. It is soluble in water to 900 ppm. The reported vapor pressure is 5.6 u (microns) at 25°C and like other thiocarbamates, Ordram is very volatile.

Ordram has an unmistakable and unpleasant odor which is due to diethyl disulfide present in the technical mixture. The pure parent compound, molinate, is essentially odorless. Molinate is unique among thiocarbamates by virture of the substitution on the trivalent nitrogen (see Figure 1). Other thiocarbamates have, for the most part, short chain aliphatic moieties on the nitrogen. In molinate, the dipropyl substitution seen in Eptam[R] (EPTC) becomes fused into a saturated cyclopolymethylene ring. This seven-member ring (hexamethyleneimine) apparently is the portion of the molecule which imparts the selective toxicity towards aquatic grasses commonly encountered in rice culture.

Environmental Degradation

Dissipation of molinate in a typical ricefield was studied by Soderquist[5] and Crosby[6]. The authors examined the processes of volatization, hydrolysis, and photolysis. These are the mechanisms primarily responsible for the environmental degradation of the compound. Since molinate does not possess a chromophore requisite of appreciable photolysis, the essential mode of dissipation is due to volatilization.

Stauffer studies[7] have confirmed this scheme and it was reported that 70% was vaporized from moist soil in 24 hours at 27°C. The loss rate could be expected to be more rapid at higher temperatures, although its solubility in water could be expected to increase as water temperature increases.

Hydrolysis of the carbamide is a secondary mechanism by which Ordram is degraded in the environment. Laboratory modeling has shown that protein-amino acid mediated photolysis may occur under natural conditions and this is borne out by the results of extensive field evaluations.

Degradation metabolites, caprolactam and hexamethyleneimine are end products of environmental photolytic decomposition of Ordram, with caprolactam being essentially stabile to further hydrolysis at pH 5-9 during a 7-day sampling period[5]. Caprolactam has a vapor pressure of 0.01 mm Hg at 20°C.

While the major degradative route for molinate is photochemical, volatilization from field water to the atmosphere is by far the major route of dissipation. Thiocarbamates are extremely volatile pesticides. Molinate was detected in the ambient air above the field, but only qualitatively, since no reliable method existed to accurately calculate the total flux of a chemical from an open field to the atmosphere[5].

MAMMALIAN TOXICITIES

Ordram has been shown to elicit a variety of biological and biochemical effects. The material presented will focus on data generated from mammalian experiments. Some of the studies performed demonstrate the phenomenon conclusively while others offer only scant evidence of a quantifiable effect. An attempt will be made to contrast, compare, and correlate the extant studies, and to draw conclusions where appropriate with the intent of extrapolation to human systems.

Metabolism

Perhaps the greatest utility will be served by first examining the metabolic transformations of Ordram in a mammalian system. Casida[8] demonstrated that S-oxidation by mixed function oxidases in the liver metabolized the parent compound to the more polar sulfoxide. Sulfoxides were also detected as transient metabolites prior to enzymatic cleavage by glutathione. It was concluded, therefore, that a primary detoxification mechanism was due to S- oxidation.

A later and more extensive study by DeBaun[9,10] reinforced data by Casida and elaborated on the suspected mechanisms by performing a balance and residue study, and a thorough identification of the urinary metabolites. 97% of an oral dose of Ordram was excreted in 48 hours by rats. Urinary excretion accounted for 88% of the radiolabeled dose with 11% detected in the feces. High and relatively constant levels of the dose remained in the blood over the seven-day period of the study. Female gonads showed a greater initial affinity for the compound and tended to void the chemical at a more rapid rate. In male rats, the half-life for ^{14}C residue departure from the gonads was approximately 4 days, as compared to about 3 days from the liver, 2.5 days from fat, and 70 days from the blood. These half-life values were calculated by linear estimate using the data of DeBaun. Unchanged Ordram accounted for only 0.1% of urinary ^{14}C. Although there are some quantitative differences, the metabolism of Ordram was qualitatively identical for male and female rats, with sulfoxidation and conjugation with glutathione being the major metabolic pathway.

The dynamics of yalan accumulation and distribution was investigated by Stepanchenko[11] who found that the compound given orally to rats at the maximum tolerated dose (300 mg/kg) accumulated particularly in the brain but also in the liver, kidney, lung, heart, and spleen. Other Soviet research[12] showed that yalan was rapidly detoxified with metabolites being found in all organs up to 24 hours post dose, and in the blood for 2 days.

Since the urinary route commanded the preponderance of metabolites and was the primary pathway of excretion, DeBaun[10] identified the compounds and quantitated their distribution. After sulfoxidation and conjugation with glutathione, a mercapturic acid derivative is formed which accounts for 35% of the urinary ^{14}C. The parent molecule is also hydroxylated at the 3 and 4 positions on the polymethylene ring and this is another major route of degradation. Hydrolysis of the sulfoxide and hydroxy derivatives yield hexamethyleneimine (15%) and 3- and 4-hydroxyhexamethyleneimine.

Acute Toxicities

As an acute poison, Ordram is only moderately toxic. Stauffer data[7] lists rat oral LD_{50} values between 500 and 800 mg/kg with a mean of 655. This compares with Soviet data[13] which determined an LD_{50} of

530 for mice and 657 and 300 for rats, both values generated by stomach injection with a maximum harmless dose of 50 mg/kg. Ordram seems to be slightly more toxic by intraperitoneal, intravenous, and inhalation routes of exposure. In the rat, acute intoxication manifests itself with ataxia, asthenia, and reduced activity(7). Chronic ingestion of 13 mg/kg was toxic to rats in a Soviet experiment(14) while 14.4 mg/kg/day showed no significant effect in the Stauffer chronic administration study.

Acute inhalation data is meager, but of the three primary routes of absorption, it appears that this is of greatest concern. Stauffer cites Dyadicheva(15) found that an inhalation dose of 2 mg/M^3 as harmless to rats, although the same paper states that the compound was moderately toxic and recommends that field workers should not be exposed to more than 0.5 mg/M^3. Stauffer's preliminary acute inhalation studies in rats and mice are being conducted and a 4-hour LC_{50} for male rats of 2.8 mg/M^3 is indicated. Toxic signs observed were depression, ataxia, salivation, aggressive behavior, hindleg weakness, and other effects. They have completed a subchronic inhalation study to examine reproductive effects(7).

Dermal sensitivity and toxicity values indicate that Ordram is not a hazard by this route of exposure. Stauffer's report gives a dermal LD_{50} in rabbits of greater than 4640 mg/kg. Their 21-day subacute evaluation with technical Ordram (6-E) showed that at 1.0 ml/kg/day for 15 doses produced 90% mortality, anorexia, diarrhea, weight loss and reversible skin irritation accompanied by a secondary infection. Dyadicheva's(13) study relates that yalan was locally irritating with cumulative effects, while Latypova(16) showed that dermal application of 0.25% or 2.5% solution of yalan did not induce any reaction in guinea pigs.

Chronic Toxicities

Subchronic and chronic parameters often give a more complete representation of the true toxic potential of an agent. Insight into longer term exposures is especially valuable since acute episodes are relatively unimportant in regard to lasting physical impairment. The insidious nature of effects due to longer term/smaller dose exposures makes precise and definitive toxicologic evaluations difficult. It is important to interpret observed manifestations of exposure in respect to organism, dose/duration, worst case estimations, pharmacology of similar compounds, and objective scientific opinion.

Saunders'(7) review of the biological effects of Ordram is organized along dose-duration lines. This review will concentrate on presenting data relative to physiological rejoinder under the assumption that the observed effects cannot be accurately delineated by arbitrary parameters such as duration.

Immunologic Effects

Among the Soviet researchers studying Ordram, Olefir, has concentrated his efforts on discerning immunologic integrity after exposures to Ordram. At 300 mg/kg in an acute study[17], he determined that the herbicide decreased serum lysozyme levels, phagocytic activity, neutrophil phagocytic index, the absorption capacity of the reticulo-endothelial system, and the skin phagocytic index. Changes in the immunobiologic reactivity occurred simultaneously with the appearance of clinical symptoms. Another acute term experiment[18] demonstrated that maximum tolerable doses of yalan increased the susceptibility of rats to infection, but had little effect on nonspecific resistance. However, in a chronic study[19], it was shown that when yalan was administered orally to rats at doses corresponding to 1/20 LD_{50} 5 times a week for 4.5 months, there was significant inhibition of nonspecific immunobiological reactivity. This inhibition lasted up to 2 months after dose was discontinued. A decrease in the resistance to a staphylococcal infection was also observed, which parallels Stauffer data generated in their subacute dermal toxicity test.

This test showed an onset of secondary infection accompanying skin irritation[7]. In a similar study, again Olefir[20] found that one month after a single administration of a maximum tolerated dose of yalan that the bactericidal activity returned to normal, and that by two months post dose the index of bactericidal activity of the skin and levels of E. coli on the skin and mucous membranes normalized. Yalan did not effect immunogenesis in mice at 1/1000 LD_{50} after 3.5 months of treatment[21]. More specifically[22], yalan decreased the formation of agglutinins in mice during immunization with typhus vaccine, but it did not appreciably lower antibody titre 1.5 months after vaccination. The serum from immune rats was able to prevent the inhibitory action of yalan. It has been shown that an inverse proportional dependance exists between the intensity of action of yalan and the complement activity of blood serum in rats[23] at toxic levels (1/20 LD_{50}) yalan inhibited the bactericidal activity of serum, but did not have effect at low dosages. Repeated oral administration of yalan at 1/20 LD_{50} for 4 months decreased the protective properties of the leukocytes, especially the ability of the neutrophils to digest phagocytized microbes[24]. This may be due to yalan's ability to cause an increase in the frequency of chromosomal aberrations and other cytogenetic effects on human lymphocytes in culture[25].

The immunological reactivity of progeny of animals exposed to yalan was also studied[26]. The effect of a 4-month treatment with 1/100 LD_{50} on the immune reactivity was a reduction in serum complement and lysozyme levels, and bactericidal activity of the skin and serum in the mother mice. Significant reductions in lysozyme levels and serum complement activity were seen in the 5-month old first generation offspring, as well as in the second litter of yalan-treated females.

These findings indicate that chronic treatment of pregnant rats with yalan reduces the activity of non-specific humoral immune factors, weakens the protective properties of the progeny's skin and mucous membranes, and their resistance to infectious agents. Yalan appears to be only a weak Type III sensitizing agent[27].

Hematologic and Hormonal Effects

Associated with immune differences are hematologic changes such as decreased blood pressure, bradycardia, and altered ECG[28], decreased hemoglobin content and hematocrit number, erythropenia, moderate leukocytosis[29, 30, 31], and acid resistance of erythorcytes[32]. Soviet data has also demonstrated deviations of normal endocrine indexes. For example, thyroid and adrenal cortex function[13, 15, 29, 33] and pituitary-hypothalamic function[34, 35] were altered. Several Stauffer studies recorded increased thyroid weights[7].

Dyadicheva saw disturbances in urinary 17-keto steroid excretion indicating a dimunition of adrenal cortex or ovarian function[33]. Bakhtizina[35] exposed rats to yalan from birth and found that general growth and sexual development was inhibited. Neurosecretion by the hypothalamus and pituitary gland was increased during the first two months and inhibited thereafter. Functioning of the ovary and testes were impaired in this experiment and in another chronic oral administration test[34]. Several studies have indicated that basal body temperature is lowered[13, 29] and that oxidative enzyme activities are decreased[13, 29, 31].

Mutagenic Effects

Ordram has been shown not to be mutagenic by several screens including the Ames protocol using histidine-requiring mutants of Salmonella typhimurium[36, 37]. However Kurinnyi[25] employed human blood lymphocyte cells and detected a slight cytogenetic effect in cultures treated with yalan at 0.02 and 0.2 ug/ml. Stauffer data from S. typhimurium shows no mutagenic activity with or without host-mediated metabolic activation, although a nitrosating media was not indicated.

Reproductive Effects

Perhaps the most graphic and best quantified results are from investigations into the ability of Ordram to cause various aberrations in reproductive processes. The Stauffer data reflects a rigorous inquiry into the phenomenon and it is worthwhile to review the results of all completed research in detail.

Bakhtizina found that yalan delayed sexual development in young rats[35], and caused morphological and functional changes in the gonads[34]. Likewise, Voitenko[38] administered yalan to young rats at 3.6 mg/kg/day orally for 2 months and found impaired spermatozoid function, decreased gonadal nucleic acid metabolism, and disrupted oxidative metabolic processes. After mating with undosed males, female rats poisoned with yalan showed an increase in the rate of resorbed fetuses and postnatal deaths, as well as teratogenic abnormalities in development. Estrus was not affected. The sibling compounds Tillam and Eptam in similar doses did not produce a gonadotoxic effect. The essence of the aforementioned work was corroberated by Anina[39] who orally dosed rats for 2 months and 3.6 mg/kg/day with yalan and observed decreased mobility and viability of spermatozoids. Fertility was reduced in females. A mode of action might be due to decreased levels of RNA and DNA and increased activity of RNA-ase and DNA-ase in the testicle[40, 41, 42]. The author suggests using a nucleic acid metabolic index[43] for predicting the long-range consequences of the effects of the herbicide. Of three herbicides evaluated, only yalan induced a change in the metabolic index while Tillam and Eptam did not.

Citing unpublished data, Stauffer relates[7] a subchronic study by Japanese researchers who performed a three-month experiment, dosing mice at 0, 45, 90, and 180 mg/kg/day, and rats at 0, 30, 60, and 120 mg/kg/day. Results included testicular atrophy although this effect was not obvious at the low dose levels i.e., 45 and 30 mg/kg/day. At all dose levels there was the thyroid hyperfunction observed by several other studies previously cited. A three-month study virtually identical to the ones previously mentioned was performed by Stauffer. Rats were fed levels of Ordram corresponding to 0, 35, 70, and 140 mg/kg.

Major findings were anorexia, increased thyroid weights at all dose levels, and decreased testicular and ovarian weights at the high dose level. Microscopic examination showed a dose-related increase in renal proximal tubule degeneration in males, a dose-related vacuolation of adrenal cortical cells at the mid and high doses, and gonadal atrophy in both sexes at the high dose. In a second three-month study in rats with dosages of 0, 8, 16, and 32 mg/kg/day gonadal atrophy was not observed. In a rat study involving a breeding phase, Ordram in the diet at 32, 16, and 8 mg/kg/day for seven weeks decreased reproductive performance. At all dose levels there was a decrease in the number of litters produced.

More work was done to delineate this effect. Fertility was inhibited in male rats at doses as low as 0.63 mg/kg/day. The effect may develop after 3-6 days of dosing and it is reversible. At a dose level of 5 mg/kg/day, spermatazoan motility is decreased while the incidence of abnormal spermatozoa is increased although spermatogenesis and spermatozoa maturation are not effected. As observed in other work, mean litter size was reduced at doses higher than 5 mg/kg/day and organ weight to body weight ratios of the seminal vesicles, prostate, and epididymides, but not testes, are reduced after two weeks of dosing at a level of 5 mg/kg/day.

A three-generation reproduction study in rats at 0, 0.063, 0.200, and 0.630 mg/kg/day showed no differences between control and test animals at the two lower dose levels and a minimal decrease in number of litters at the high dose. Postnatal survival was decreased at 0.63 mg/kg/day. Stauffer selected 0.2 mg/kg/day as the no-effect level.

Even though interference with reproductive mechanisms by oral exposure are clearly suggested with the previously referenced works, more intriguing results are observed in Stauffer subchronic inhalation reproduction studies. It can be assumed that this route of exposure most realistically models conceivable environmental or occupational exposure since continued oral ingestion or external skin contact with the compound is unlikely for reasons which are obvious.

Stauffer protocol used groups of 20 male and 10 female rats and exposed them for 6 hours a day, five days per week for 13 weeks to atmospheres containing 0, 2.2, 11.1, and 42 mg/M^3 of Ordram. One-half of the males were mated to naive, untreated females after 1 and 3 months of exposure and after 1 and 3 months of non-exposure. Male reproductive performance was reversibly impaired at all doses. Those parameters which could be quantitated included: reduced mean number of implants/increased resorption of fetuses and reduced number of fetuses per female. Post exposure breeding results were comparable to controls. At the end of the exposure period, mild focal degeneration of the testes was observed in 40% of the low dose rats and 30% of the mid-dose rats. Severe extensive testicular degeneration was found in 80% of the high dose rats. Additionally, the epididymides of 80% of the high dose rats contained abnormal spermatozoa as did 30% of the mid-dose and 20% of the low dose rats. Sperm counts were lowered in 60% of the high dose and 10% of the low dose rats.

Pituitary weights were altered at the high dose, and at the mid-dose as were adrenal and thyroid weights. At the end of the post-exposure, the mean testicular weight for the high dose males was significantly reduced as compared to normal weights.

Acute inhalation studies in rats and mice are or have been conducted by Stauffer with Ordram. Most signs appear to be dose-related in severity and duration.

SUMMARY

Ordram is a thiocarbamate herbicide used to control aquatic grasses. Workers are exposed to Ordram in a variety of occupational situations. The herbicide has been shown to cause a reversible infertility in male mice and rats in some screens at doses of 1/1000 of the oral LD_{50}.

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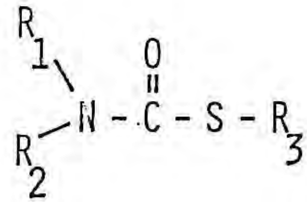
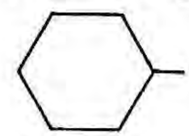
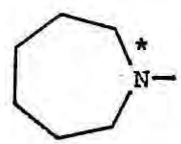
FIGURE 1

COMMON NAME, CHEMICAL NAME, AND CHEMICAL STRUCTURE OF THE THIOCARBAMATE HERBICIDES (1, 2)



COMMON NAME	CHEMICAL NAME	R_1	R_2	R_3
Butylate	S-ethyl diisobutylthiocarbamate	$CH_3 - \overset{CH_3}{\overset{\vert}{CH}} - CH_2 -$	$CH_3 - \overset{CH_3}{\overset{\vert}{CH}} - CH_2 -$	C_2H_5 -
Cycloate	S-ethyl N-ethylthiocyclohexane carbamate	C_2H_5-		C_2H_5-
EPTC	S-ethyl dipropylthiocarbamate	C_3H_7-	C_3H_7-	C_2H_5-
Pebulate	S-propyl butylethylthiocarbamate	C_2H_5-	C_4H_9-	C_3H_7-
Molinate	S-ethyl hexahydro-1H-azepine-1-carbothioate			C_2H_5-
Diallate	S-(2,3-dichloroallyl) diisopropylthiocarbamate	$CH_3 - \overset{CH_3}{\overset{\vert}{CH}} -$	$CH_3 - \overset{CH_3}{\overset{\vert}{CH}} -$	$Cl-\overset{H}{C}=\overset{Cl}{C}-CH_2-$
Triallate	S-(2,3,3-trichloroallyl) diisopropylthiocarbamate	$CH_3 - \overset{CH_3}{\overset{\vert}{CH}} -$	$CH_3 - \overset{CH_3}{\overset{\vert}{CH}} -$	$Cl-\overset{Cl}{C}=\overset{Cl}{C}-CH_2-$

* The nitrogen atom in the molinate ring structure is the nitrogen atom of the parent structure.