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The Acute Inhalation Toxicology of the Technical Grade Organoarsenical Herbicides, Cacodylic Acid and Disodium Methanearsonic Acid; A Route Comparison

James T. Stevens[1], Louis C. DiPasquale[2], and Jackie D. Farmer
U.S. E. P. A., Research Triangle Park, N.C. 27711

Organoarsenicals have played an important role in medicine as trypanocides and in agriculture as insecticides and herbicides (FROST 1967). Cacodylic acid (hydroxydimethylarsine oxide, $(CH_3)_2AsO_2H$) and disodium methanearsonic acid (DSMA, $CH_3AsO_3Na_2$) have been used with good success as contact herbicides, as cotton defoliants and as nonselective contact herbicides on noncrop areas.

Despite the fact the precursor for their synthesis, arsenic trioxide, is highly toxic to rats given perorally (HARRISON et al. 1958), little information is available concerning the acute toxicity of these organoarsenicals. Acute inhalation studies are completely lacking.

This report presents the results of an acute inhalation toxicity evaluation of two technical grade organoarsenicals, Phytar® 138 and Ansar® 8100. The respiratory irritancy potential of these compounds, and a comparison of acute toxicity of these organoarsenicals to the mouse and rat after intraperitoneal administration are also provided.

MATERIALS AND METHODS

Animals. Adult Swiss-Webster mice (25-30) obtained from Hilltop Lab Animals, Inc., Scotdale, Penn.[3], or age-matched adult Sherman rats (100-200 g) obtained from a barrier colony maintained by the Communicable Disease Center, Atlanta, Georgia were used in all experiments. Before and after treatment rats were held in a laminar

[1]Present address CIBA-GEIGY Corporation, P. O. Box 11422, Greensboro, North Carolina 27409.
[2]Present address Gillette Medical Evaluation Laboratories, 1413 Research Boulevard, Rockville, MD 20850.
[3]Mention of commercial products does not imply endorsement by the United States Environmental Protection Agency.

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flow room maintained at 22 ± 1°C, 55 ± 5% relative humidity and on a 12 hour light cycle day; mice were held in a conventional animal room under similar conditions. Animals were fed Purina Chow and water ad libitum.

Inhalation Exposure. All exposures were conducted as whole body in a 120 liter plexiglass chamber with air flow of 10 liters/min. Clean, dry air produced by a non-cycling refrigerated air dryer was used for chamber and generation supply.

All animals were exposed to either Phytar® 138 (65.6% cacodylic acid), Fisher Purified Cacodylic acid (95.5%) or Ansar® 8100 (80.1% disodium methanearsonic acid). All reported concentrations of organoarsenical are expressed as active ingredient.

Ten animals of each sex were exposed to aerosols of technical cacodylic acid and disodium methanearsonic acid generated as a dust using the Farmer Pulse-Puff Fluidizing Dust Generator as previously described (DIPASQUALE et al. 1976). This generator was designed as a a fluidizing bed which serves the functions of de-agglomeration of dust to be generated and a capacitor-like effect in damping any fluctuations arising from discontinuous flow of dust to the exposure chamber.

Analysis of Atmosphere. Particle size analyses were determined at least twice during an exposure using a Royco® 225 Particle Monitor with a 508 Multichannel Analyzer Module (Royco Instrument, Inc.) with a sensitivity to 0.3 micrometers.

Particle size is expressed as mass median diameter of particles computed by probit analysis, and extrapolation, using the Hatch equation (HATCH and CHOATE 1929); Chamber concentration was assessed gravimetrically by collecting a sample for 2 min at a rate of 5 liters/min onto a millipore filter (.45μ pour size), and weighing on a Mettler H31 balance. Samples were taken at 15 min intervals during exposure.

Observations. Animals were observed during exposure and for 14 days after exposure. The inhalation lethal concentration killing 50% of the animals (LC_{50}) was determined at 14 days post exposure (LITCHFIELD and WILCOXON 1949). Gross pathological evaluation was conducted on animals dying within the 14 day period; survivors were necropsied at 14 days.

Whole-Body Plethysmography. Adult male Swiss-Webster mice were subjected to five-minute exposures to dust aerosols of the organoarsenicals. Exposures were conducted after the method previously described (ALARIE 1966). Changes in respiratory rate were measured using Model 377 pressure transducers and recorded on a Model 350 Electronic Recording Module (Harvard Apparatus Co., Millis, Massachusetts). Pesticide was generated using the Farmer Pulse-Puff Fluidizing Dust Generator. Aerosols were generated from Ansar® 8100, Phytar® 138, or Ansul purified cacodylic acid (99.5%).

The measurement of effects from these studies was based upon the decrease in the frequency of individual mouse respirations caused by an increase in the duration of the expiratory phase of the breathing cycle. Three mice were used for each determination and several concentrations tested to define the concentration which results in a 50% decrease in respiration rate (RD_{50}). RD_{50} values were calculated using linear regression analysis.

Other Routes of Administration. Adult Sherman rats and Swiss Webster mice were given Fisher Purified cacodylic acid (95% cacodylic acid and 5% moisture) or Ansar® 8100 by intraperitoneal/administration. Female Sherman rats were also administered purified cacodylic acid via the tail vein. All animals were given doses adjusted for body weight and dose level using an aqueous stock solution of 500 mg/ml with the volume not exceeding 1.5 ml. The level of dosing ranged from 400 to 625 mg/kg. LD_{50} values were calculated as previously described (LITCHFIELD and WILCOXON 1949).

RESULTS

Inhalation Exposures. Rats and mice were exposed to very high concentrations of organoarsenicals for 2 hrs. Particulate concentrations expressed in terms of the technical materials generated were in excess of 10 and 8.6 mg/liter for Phytar® 138 and Ansar® 8100, respectively. Chamber atmospheres at these concentrations were so dense that it was difficult to clearly visualize animals for pharmacotoxic responses.

Cacodylic acid produced deaths in rats (TABLE 1). Two out of ten males succumbed after exposure to 4.1 ± 0.56 mg/liter during the 14 day observation period; however, none of the males died after exposure to a concentration of 6.9 ± 0.25 mg/liter. Therefore, it was not possible to compute an LD_{50} value for male rats exposed to cacodylic acid.

TABLE 1

Results of two hour exposures of rodents to dust atmospheres of organoarsenicals.

Organo-arsenical	Species	Sex	No. Of Animals	Maximum Exposure Level (mg/liter)	MMD (μm)	Estimated LC_{50} mg/liter
Cacodylic acid[a]	Rat	M	30[b]	6.9±0.25[c]	3.9[d]	>6.9
		F	40	6.9±0.25	3.4	3.9
	Mouse	M	10	6.4±0.33	4.6	>6.4
		F	10	6.4±0.33	4.6	>6.4
DSMA[a]	Rat	M	10	6.1±0.65	3.7	>6.1
		F	10	6.1±0.65	3.7	>6.1
	Mouse	M	30	6.9±0.32	3.1	>6.9
		F	30	6.9±0.32	3.1	>6.9

[a] Phytar® 138 used for exposures (65.6% cacodylic acid); Ansar® 8100 (80.1% DSMA).

[b] Ten animals per sex exposed at each level.

[c] Maximum chamber concentration to which the animals were exposed; mean ± standard error (N=8) for gravimetric samples taken at 15 minute intervals during exposure.

[d] Mass Median Diameter in micrometers.

Exposure of female rats to mean concentrations of 4.0 ± 0.15, 4.1 ± 0.56 and 6.4 ± 0.33 mg/liter produced mortality rates of 40%, 70% and 90%, respectively. Therefore, the LC_{50} for female rats exposed to cacodylic acid was computed to be 3.9 (2.9-5.3) mg/liter with a slope of 1.21 (1.07-1.70). In addition, one male mouse exposed to 6.4 ± 0.33 mg/liter died during the 14 day observation period.

Rats and mice exposed to dust atmospheres of cacodylic acid exhibited respiratory distress, rhinorrhea and porphyrin-like encrustation of the eyes during exposure. Post exposure diarrhea and a decreased weight gain was also noted. All female rats exposed to 6.94 mg/liter of Cacodylic acid exhibited erythematous lesions on feet and ears with a reddish tinge to the fur. Female rats which died during the 14 day post-exposure period did not exhibit any consistent pattern of gross pathology, although evidence of impacted caecum, and a mucus-like material and blood in the intestine was observed. Bright red lungs and dark spots on the lungs were also noted.

Rats and mice exposed to atmospheres of DSMA were observed to have respiratory distress during exposure but recovered rapidly after removal from the exposure chamber. No mortality occurred in either species.

No gross organ lesions were observed during necropsy of rats and mice surviving exposure to cacodylic acid or DSMA held for the period of 14 days.

Respiratory Irritancy Potential. The results of tests to evaluate the respiratory irritant potential of these organoarsenical pesticides is shown in TABLE 2.

Both DSMA and cacodylic acid decreased the respiratory frequency of mice as a result of exposure to each of the pesticide dust atmospheres. DSMA produced a more pronounced effect than cacodylic acid. The RD_{50} for DSMA was 1.54 mg/l compared to 3.15 mg/l for purified cacodylic acid. The same magnitude of response was observed with purified cacodylic acid and Phytar® 138 at equal concentration of organoarsenical. High concentrations of diatomaceous earth ran as a positive control produced minimal response.

TABLE 2

Respiratory irritant potential of organoarsenical pesticide dusts as determined by mouse whole-body plethysmography

Compound	N	Corrected Concentration (mg/liter)	MMD(μm)	Respiratory Rate (% Decrease)[c]	Calculated RD_{50} (mg/liter)
Ansar® 8100 (DSMA)	3	0.163	--[b]	5.7	
	3	0.336	11.1	22.5	1.54
	3	0.769	--[b]	33.0	
	3	2.136	5.4	63.8	
Phytar® 138	3	1.154	<1.0	23.4	
Cacodylic Acid[a]	3	0.174	7.8	11.1	3.15
	3	1.015	--[b]	24.3	
	3	2.070	2.6	35.7	
Diatomaceous Earth	3	3.516	<10.0	12.6	

[a] Purified cacodylic acid, 99.5%.

[b] Mass Median Diameter not determined.

[c] Mean of at least 3 animals.

To determine the significance of the weak irritancy response of cacodylic acid on the computed LC_{50} value after inhalation exposure, rats were subjected to an atmospheric concentration of respirable aerosol (MMD=5.4 μm) of Phytar® 138 of 16.5 ± 1.6 mg/liter (10.8 ± 1.1 mg/liter, cacodylic acid) for two hours. The estimated RD_{50} in the mouse was determined to be 3.15 mg/liter, assuming no species difference in irri-

tancy response, rats were subjected to 3.4 times the RD_{50}. Exposure to 4.1 ± 0.56 mg/liter resulted in 20% and 70% mortality in male and female rats, respectively; exposure to 10.8 ± 1.1 mg/liter only produced 10% and 40% mortality in male and female rates, respectively.

Other Routes of Administration. The results of intraperitoneal administration of organoarsenicals is presented in TABLE 3.

TABLE 3

Comparison of LD_{50} values and their slopes for rodents after intraperitoneal administration of organoarsenicals

Compound	Species	Sex	No. Of Animals	LD_{50}[c] (95% Confidence Limits)	Slope (95% Confidence Limits)
Cacodylic acid[a]	Rat	M	30	720 (637-814)	1.20 (0.89-1.62)
	Rat	F	56	520 (481-637)	1.18 (1.02-1.37)
	Mouse	M	80	520 (491-551)	1.13 (0.96-1.33)
	Mouse	F	80	600 (561-642)	1.10 (0.83-1.45)
DSMA[b]	Rat	M	30	600 (550-792)	1.43 (0.91-2.26)
	Rat	F	30	561 (449-701)	1.44 (0.81-2.56)
	Mouse	M	24	600 (556-648)	1.11 (0.76-1.79)
	Mouse	F	24	681 (625-742)	1.17 (0.63-1.92)

[a] Cacodylic acid used was Fisher® Purified (95%); LD_{50} calculated on the basis of cacodylic acid present.

[b] Ansar® 8100 (80.1% disodium methanearsonic acid); LD_{50} calculated on the basis of DSMA present in Ansar® 8100.

[c] LD_{50} and 95% confidence limits computed using the method of LITCHFIELD and WILCOXON (1949); data expressed in mg/kg.

Cacodylic acid was found to be more toxic to female rats and male mice after intraperitoneal administration than to male rats and female mice. No differences in toxicity were noted between sexes or species for DSMA. In addition, the slopes of mortality data were not different for any of the comparisons made for these organoarsenicals.

Besides evaluating the toxicity after intraperitoneal dosing, female rats were given cacodylic acid intravenously for comparative purposes. The intravenous LD_{50} was determined to be 470 (432-512) mg/kg for 36 animals, having a slope of 1.21 (0.86-1.53). These values were not different from those obtained after intraperitoneal administration.

Similar signs were noted after intravenous and intraperitoneal administration as seen after inhalation exposure. If the animals died, death occurred within the first 4 days post exposure. Signs included rough fur, difficult breathing, assumption of the fetal position, loss of the righting reflex, loss of body temperature and rigidity. Gross observations included small red thymus, bright lungs, dark adrenals, livers, and spleens, impacted caecum, evidence of irritation of the stomach lining, blood in the intestine and mucus-like material in the intestine.

DISCUSSION

Only the female Sherman rat exhibited sufficient inhalation toxicity to calculate an LC_{50} value; then only after exposure to cacodylic acid. The reason for the greater sensitivity of the female rat to inhalation exposure to cacodylic acid is not known at this time.

Compounds eliciting RD_{50} values greater than 0.50 mg/liter are not considered significant irritants (ALARIE personal communication). Cacodylic acid did not effect a 50% reduction in respiratory rate at 2.07 mg/liter, and had an estimated RD_{50} of 3.15 mg/liter; the calculated RD_{50} for DSMA was 1.54 mg/liter. HATCH and GROSS (1964) have proposed that deposition of particles in the respiratory tract increases as the respiration rate decreases as a consequence of increasing the potential for gravitational settling of particles in an air stream whose transit time has been lengthened. Accompanying the increase in particle deposition would be an increased inhalation hazard. This premise is not supported by the fact that an exposure to a level of 10.8 $\pm$ 1.1 mg/liter resulted in fewer deaths than 4.1 $\pm$ 0.56 mg/liter of cacodylic acid.

The lack of marked differences in the toxicity of these two structurally similar organoarsenicals administered intraperitoneally would suggest that the irritancy potential of these compounds may interfer with the determination of their inhalation toxicity.

STEVENS *et al.* (1977) have shown that ^{14}C-Cacodylic acid is rapidly absorbed from the lungs after intratracheal instillation. The exposures presented in this paper were conducted with highly respirable aerosols. Indeed, the pharmacotoxic signs and gross lesions observed were very similar after inhalation, intraperitoneal and intravenous administration. These facts suggest that failure to observe significant inhalation toxicity was not due to the inability of the aerosols to achieve systemic effects.

SACHSSE et al. (1973), after comparing 1 and 4 hour exposures to pesticides, suggested a new categorization of inhalation hazard assessment based on a 4 hour exposure period. The results presented in this paper support the need for increasing the exposure period. Prudence would dictate if high concentrations are required to produce mortality, and irritancy potential has been indicated, that the duration of exposure should be lengthened so that the level of exposure can be reduced to one that does not result in significant respiratory effects, yet enable the investigator to meet the safety requirements.

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