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INDUSTRIAL HYGIENE FOUNDATION OF AMERICA, INC.

MELLON INSTITUTE <> 4400 FIFTH AVENUE

PITTSBURGH 13, PA.

Report on: A COMPARISON OF THE SUBACUTE VAPOR TOXICITY OF CHLOROTHENE NU, TRI-ETHANE, TYPE 314 AND TRI-ETHANE, TYPE 324

For: PITTSBURGH PLATE GLASS COMPANY

Date: MAY-SEPTEMBER, 1965

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May-September, 1965

by

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INTRODUCTION

This report presents data and conclusions on the subacute toxicity of three chlorinated hydrocarbons, Chlorothene NU (189-3350), Tri-Ethane, Type 314 (189-3351), and Tri-Ethane, Type 324 (189-3352). The objective of the study was to determine the vapor toxicity of these materials, as revealed by behavioral, growth, mortality, and pathological data, on laboratory animals exposed daily to the solvent vapors for a four-week period. The effects were evaluated by comparing the response of groups of guinea pigs subjected to the vapors of the test materials with the response on guinea pigs exposed to air, alone.

MATERIALS AND METHODS

The following materials were received for testing:

(1) Chlorothene NU, Lab. Ref. No. 189-3350.
(2) Tri-Ethane, Type 314, Lab. Ref. No. 189-3351.
(3) Tri-Ethane, Type 324, Lab. Ref. No. 189-3352.

The apparatus consisted of four, seven-cubic-foot, stainless steel chambers, three vapor generating units, and analytical chemical equipment. Each chamber contained five animal cages, had a glass-windowed door for observation of the animals during exposure, and was constructed to maintain the animals twenty-four hours per day. Each vapor-generating unit was composed of a monodrum mechanism(1), a single syringe feeder(2), a glass Graham condenser, and glass and tygon tubing connections. The monodrum mechanism drove the single syringe feeder, which contained the

solvent, at a calculated, constant rate, delivering the required amount of chemical into the condenser. Hot water was passed through the outer jacket of the condenser and as the solvent dropped into its tubular center, it evaporated. The vapors were mixed with air and drawn through the chamber. The delivery of each of the chlorinated hydrocarbons and the air flow through the respective chambers was adjusted so that the concentration in each chamber was approximately 1,000 parts per million. The calculated vapor concentrations were in good agreement with the results of analyses obtained by combustion and colorimetric determination of total halogen using mercuric chloranilate[3].

Three groups of 15 female, albino guinea pigs, each, were exposed to the three materials, respectively, while a fourth control group of 15 guinea pigs was exposed in a similar chamber to air. All of the guinea pigs were fed Wayne Guinea Pig Feed ad libitum except during exposure when feed and water were withheld. The guinea pigs were weighed each morning before exposure. The animals were exposed seven hours per day, five days per week, for four weeks. All of the guinea pigs were observed during and between exposures for signs of toxicity. Animals that died during the testing period and those sacrificed terminally were autopsied.

The livers and kidneys were dissected and fixed in formalin following the determination of the liver and kidney weights. The lungs, after dissection, were distended by formalin-fume fixation at a pressure of eight cm. of water. The lungs, liver, and kidneys of all guinea pigs of each group were subjected to histopathological preparation and examination.

RESULTS

The observed data are summarized in Table 1 and the time progression of body weights showing the average and upper and lower limits is shown in Graph 1.

Table 1 points out the level of significance of differences between exposed and control animals. The exposure conditions produced no visible evidence of toxic signs or behavioral changes either during or between exposures. Also, the apparent differences in weight gain of exposed animals as compared to controls which were noted by the end of the exposure series seemed to be minimized somewhat during the several days which passed before autopsy.

The comparison of individual weight gains, also shown in Table 1, was more highly significant in a statistical sense than were the group averages for final body weight.

Pathologic Examination:

Gross Findings: Except for the presence of obvious pneumonia in several of the animals no significant abnormality was found.

Microscopic Findings:

Lungs: There was some degree of chronic pneumonitis in nearly all guinea pigs, the ones exposed to the vapors of the test substances as well as those of the controls. This was characterized by proliferation of alveolar cells and mural thickening as well as atelectasis of the involved regions. The cause of the atelectasis was probably related to inadequate active surfactant

production by the altered alveolar cells. In addition, there were some animals in each group that had a superimposed acute pneumonia. Most of these acute pneumonias were slight; although a few animals with very severe and extensive involvement were noted. Inasmuch as the different experimental groups were not involved to a more severe degree than the control group by the chronic pneumonitis or by the superimposed acute pneumonia, it is a reasonable conclusion that this pulmonary disease was of spontaneous origin and unrelated to the vapor exposures.

The question arises however, whether or not the presence of the spontaneous disease in the lungs of these animals would interfere with the recognition of changes that may have been produced by the inhalation of the chemical vapors. The answer to this question is a definite "no". One would expect those portions of the lungs not affected by spontaneous disease to show the effects of the vapors if the latter had been capable of irritating the lung tissue in the concentrations employed. Such was not the case however. Those portions of the lungs not affected by spontaneous disease showed no evidence of irritation by the vapors. Furthermore, these animals, had a reduced pulmonary reserve, and they were therefore much more sensitive test subjects than normal animals would have been. On the other hand, if the chemical would have had a deleterious effect on the lungs and had caused some of the animals to die, it would have been very difficult to evaluate the contribution made by the vapor exposure and that made by the spontaneous disease.

Liver: No significant abnormality was found microscopically in any of the livers except for one control animal in which a moderate amount of fatty degeneration was present. However, this animal also had a very severe bronchopneumonia with large pulmonary abscesses and the fatty degeneration was, in all probability, secondary to the latter disease.

Kidneys: A number of animals had small foci of chronic pyelonephritis, inclusive of one control. There was no evidence of the degenerative changes that are characteristic of those produced by chlorinated hydrocarbons.

DISCUSSION

The exposure conditions which existed in this series of experiments were not identical with the conditions studied by Torkelson, et. al. [4], on inhibited 1, 1, 1, -Trichloroethane (Chlorothene), so no direct comparisons should be made. However, our results do supplement and support these earlier studies.

The death of a single animal in the control and the Tri-Ethane Type 324 groups is not considered significant and was probably due to bacterial disease.

All of the chlorinated hydrocarbon vapors produced a decreased rate of growth as compared to controls. This trend was also noted by Torkelson. The low average for the Chlorothene NU group was brought about by the large contribution of 1 or 2 animals which actually lost weight during the exposure. By and large, however, the majority of animals exposed to Chlorothene NU showed the same response pattern as did the animals exposed to the other chlorinated vapors.

The examination of growth patterns is a common index of stress in animal experiments. The changes noted here are evidence of stress caused by the exposures; however, the tendency to conjugate over-exposures have ceased indicates they are of little or no physiological significance.

The absolute liver weights of animals exposed to Chlorothene NU and Type 314 Tri-Ethane were less than those of the controls or Type 324 Tri-Ethane exposed animals. This effect may be explained by the lesser rate of overall growth of the animals since the liver weight/body weight ratios showed no significant changes.

The absolute kidney weights of animals exposed to Type 314 Tri-Ethane were somewhat depressed but again the kidney weight/body weight ratios showed this effect to be due to growth pattern. The apparent increase in kidney weight/body weight ratio for Chlorothene NU, while statistically significant compared to either controls or other exposed animals, may have been due to either the depressed rate of growth (or even loss in weight) or to chance. Since Torkelson found no significant changes in kidney weight/body weight ratios in any of his studies, we would not wish to place any importance on this finding unless confirmed by additional experimentation.

The absence of microscopic evidence of damage to the lungs, liver, or kidneys as a result of these exposures indicates that the observed changes have little physiological significance and given sufficient recovery time all observed effects would probably have returned to normal. Body weight measurements tend to support this argument, for in three to five days after exposures were stopped, the significance of the difference between exposed and control animals had become considerably less.

There were no significant differences found as to the comparative effects of the two Tri- Ethanes.

CONCLUSIONS

The major effect noted from exposure of guinea pigs for seven hours per day, five days per week for four weeks to vapors (approximately 1,000 ppm) of three chlorinated solvents was a lessened rate of growth during the exposure interval which showed evidence of a tendency to compensate rapidly after exposures were terminated. An apparent elevation of the kidney weight/body weight ratio of animals exposed to Chlorothene NU is considered due to chance since all other criteria of effect were compatible with results found by Torkelson.

There were no fatalities attributable to the exposures. There was no apparent microscopic evidence of damage to lungs, liver or kidneys caused by the exposures.

There was no evidence of any comparative differences in effect caused by exposure to either Tri-Ethane, Type 314 or Tri- Ethane, Type 324.

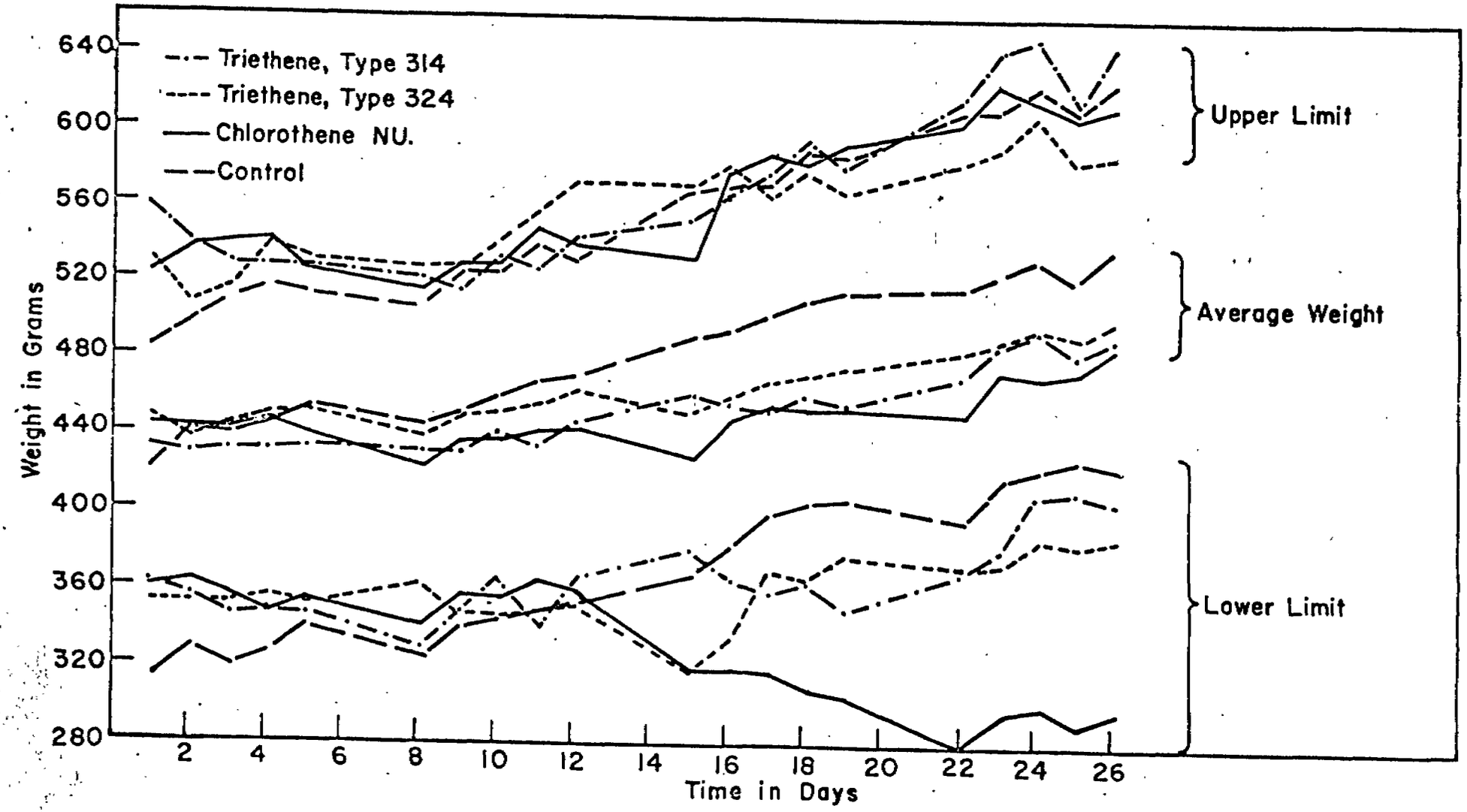
REFERENCES

(1) Monodrum Apparatus manufactured by Gorrell and Gorrell, Westwood, N.J.

(2) Single-Syringe-Feeder manufactured by Modern Metalcraft, Midland, Mich.

(3) Method for the Determination of Chloride Using Mercuric Chloranilate. Fisher Scientific Company Technical Data Sheet.

(4) Torkelson, T. R., Oyen, F., McCollister, D. D., and Rowe, V. K.: Toxicity of 1,1,1,-Trichloroethane as Determined on Laboratory Animals and Human Subjects. Am. Ind. Hyg. Assoc. J., 19: 353-362, (Oct. 1958).



GRAPH 1
EFFECT OF MATERIALS ON GROWTH

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Table 1--Effects[1] of four week Vapor Exposure of Guinea Pigs to three Chlorinated Solvents (1,000 ppm, 7 hours/day, 5 days/week)

Effect	Chlorothene NU	Tri-Ethane (Type 314)	Tri-Ethane (Type 324)	Control (Air Exposed)
Mortality	0/15	0/15	1/15(11th day)	1/15(25th day)
Initial Body Wt. (gms.)	444(362-524)	434(366-559)	448(355-531)	423(314-484)
Final Body Wt.[2] (gms.)	486(289-615)[3]	492(408-646)[3]	501(389-589)[4]	539(427-629)
Individual Wt. Gain[2] (gms.)	35(-118-106)[5]	59(12-109)[5]	57(7-105)[5]	112(47-171)
Liver Wts. (gms.)[2]	21.1(14.6-27.0)[4]	20.8(12.0-26.2)[4]	23.6(12.5-32.6)[6]	24.0(16.3-35.7)
Liver Wt./Body Wt. (%)[2]	4.2(2.4-5.4)[6]	4.2(2.9-5.5)[6]	4.6(3.0-6.4)[6]	4.5(3.4-6.5)
Kidney Wts. (gms.)[2]	4.16(2.84-4.96)[6]	3.74(3.18-4.52)[3]	4.07(3.18-4.84)[6]	4.26(3.62-5.05)
Kidney Wt./Body Wt. (%)[2]	0.86(0.68-1.01)[3]	0.77(0.61-1.00)[6]	0.79(0.67-0.98)[6]	0.79(0.68-0.90)

(1) Mean Values with observed range in parentheses.
(2) Not counting animals which died.
(3) Significantly different from controls at p=0.1
(4) " " " " " p=0.15
(5) " " " " " p=0.001
(6) No significant difference " " " p=0.2