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TS

OUNDS. *Carey*
rl E. Heussner. 243
II. STUDIES OF
mas R. Sweeney
255
ENINGS UNDER
259
SLOTS. *Leslie*
267
MAGNESIUM.
277
E GLYCOLS OF
). *Henry F.*
Jane Seaton
281
WITH RADIO-
285
ko and John
289
292
293
171

WATER_PCB-SD0000063135

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THE RESPONSE OF LABORATORY ANIMALS TO MONOMERIC STYRENE*

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MONOMERIC styrene (phenyl ethylene) is a colorless liquid with a boiling point of 144.7–145.1°C., a melting point of −30 to −31°C., and a vapor pressure of 4.3 mm. Hg at 15°C. This compound polymerizes very easily, particularly at high temperatures. A knowledge of the physiological effects produced by monomeric styrene is desirable since large quantities are being used in the manufacture of plastics and synthetic rubber.

In conducting the vapor experiments, small quantities of monomeric styrene were obtained fresh from the production still each week. 4-tertiary-Butyl catechol was added to this fresh material to the extent of 0.01% for the purpose of inhibiting polymerization. As an added precaution the measuring pumps were housed in refrigerated boxes and kept at 0 to 5°C. In the experiments on rat toxicity and skin irritation fresh material without inhibitor was used. All of the monomeric styrene used in this experimental work had a purity of 99% or better.

SINGLE VAPOR EXPOSURES

Rats and guinea pigs received single exposures of various lengths of time to a series of concentrations in order to determine, for each concentration, the shortest exposure to which all succumbed and the longest exposure which all survived. After the single exposure the animals were observed for a period of 2–4 weeks. Tissues were obtained from representative animals for microscopic examination.

* Received for publication June 23, 1942.

The apparatus and experimental procedure used in this work have been described previously (1).

The results obtained from work with 405 rats and 410 guinea pigs are given in Table 1 and Figures 1 and 2.

The first reaction shown by all the animals receiving single exposures was eye and nose irritation as evidenced by lachrymation, salivation, and nasal discharge as well as by violent scratching and rubbing of the eyes and nose. This irritation was extremely severe in the case of the higher concentrations. No other response was shown by rats and guinea pigs exposed to 6.0–6.3 mg./l. except a general weakness and unsteadiness which gradually developed after 12–30 hours. This general debilitation became more evident in those animals exposed to 9.3 mg./l., even becoming quite marked after 24–30 hours, with actual loss of consciousness on the part of a few of the exposed animals. Both rats and guinea pigs exposed to 11.6 mg./l. showed varying degrees of weakness and stupor followed by incoordination, loss of equilibrium, tremors, and finally unconsciousness after 10 to 12 hours. In the case of 23.2 mg./l., the animals became weak and unsteady almost immediately upon exposure, and soon lost their equilibrium, falling upon their sides. While in this "side position" the animals made running movements with their legs and often showed tremors and clonic convulsions. Usually within an hour these animals were completely unconscious. This same sequence of symptoms occurred somewhat more quickly in the animals exposed to 46.4 mg./l.

These results show that aside from its local

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irritant action, the primary effect of monomeric styrene vapor is upon the central nervous system, producing incoordination, tremors, loss of equilibrium, and finally complete loss of consciousness.

TABLE I

SUMMARY OF SINGLE EXPOSURES TO MONOMERIC STYRENE VAPOR

CONCENTRATION		RATS		GUINEA PIGS	
mg./l.	p.p.m. (approx.) 25°C. 745 mm. Hg.	100% survival exposure (hours)	100% lethal exposure (hours)	100% survival exposure (hours)	100% lethal exposure (hours)
6.0–6.3	1,300	30	>40	16	40
9.3	2,000	16	>30	7	30
11.6	2,500	8	21	5	14
23.2	5,000	2	8	3	8
46.4	10,000	1	3	1	3

grees of leucocytic infiltration. Under comparable conditions of exposure, the pulmonary damage was more severe in the guinea pigs than in the rats.

The degree of acute pulmonary damage varied in general with the vapor concentration of styrene and the length of exposure. Marked pulmonary lesions were produced at all concentrations studied when the length of exposure was such that at least some of the exposed animals died following the exposure.

The only other organs that were affected by single exposures to styrene were the liver and kidney; however, only a comparatively few animals showed changes in these organs and even in these cases the lesions were not severe or persistent, but rather they were the frequently occurring acute parenchymatous changes commonly associated with many acute intoxications. These liver and

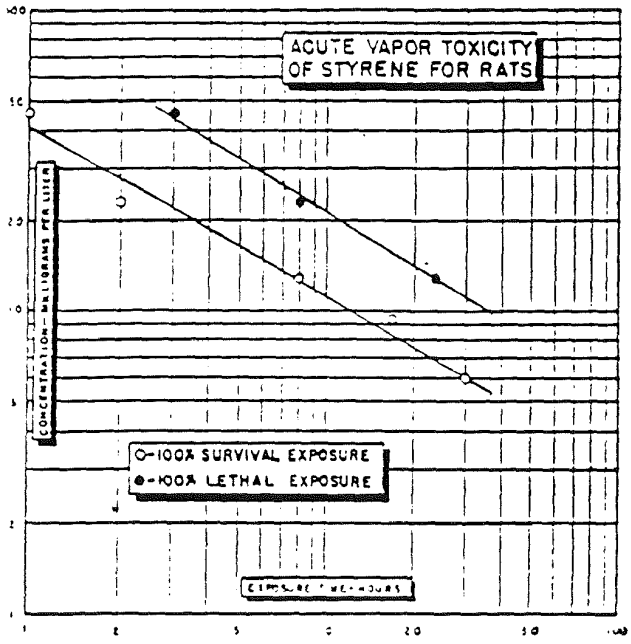

FIG. 1

The outstanding lesions that were encountered in animals that had received single exposures to monomeric styrene were found in the lung. These pulmonary changes varied from slight congestion to the most marked acute response to a severe irritant, characterized by congestion, frequent hemorrhage, edema, exudation, and varying de-

kidney changes were more frequently found in the rats than in the guinea pigs. Such changes were most often produced by a single exposure to [illegible] mg./l., were rarely produced at the higher concentrations, and infrequently at the lower concentrations.

No doubt most of the deaths that occurred

ring the single exposures were due to the primary action of monomeric styrene upon the central nervous system, while many of the delayed deaths resulted from the pneumonia which frequently developed following acute lung irritation.

Repeated Vapor Exposures

5 milligrams per liter (approx. 1,000 p.p.m.)

As a preliminary experiment, 28 rats, 12 guinea pigs, and 2 rabbits were given repeated 7½ to 8-hour exposures, 5 days a week, to 9.3 mg. L. without producing significant physiological response. Six to 6.3 mg. L. was selected as the next concentration for investigation.

6.0–6.3 milligrams per liter (approx. 1,000 p.p.m.):

A total of 50 rats, 24 guinea pigs, 12 rabbits, and 4 monkeys were given repeated 7 to 8-hour exposures to 6.0–6.3 mg. L., 5 days a week over a period of at least 6 months. Regular weight records and observation notes were kept on all the exposed animals and on a similar number of con-

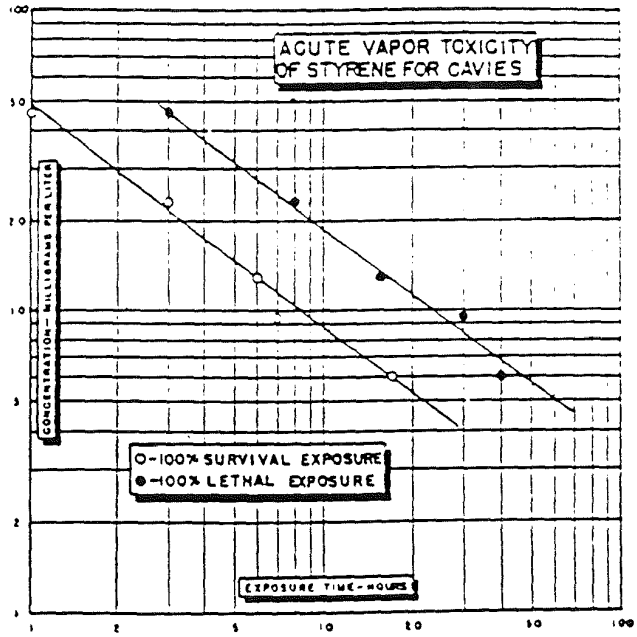

FIG. 2

Rats received up to 105 exposures, guinea pigs up to 98 exposures, and rabbits up to 126 exposures.

The rats and guinea pigs showed marked eye and nose irritation during the exposures. Throughout the experiment these animals appeared listless, dirty and unkempt, and made very poor weight gains as compared with the controls. The rabbits were apparently not affected by these repeated exposures.

The results of this experiment indicate that, although rats and guinea pigs can survive many repeated exposures to 9.3 mg./L., this concentration of monomeric styrene is definitely higher than that which can be tolerated for repeated exposures trols. Frequent blood examinations were made on a representative group of rats and rabbits and upon all the monkeys. At the end of the experiment, tissues were saved from many of the animals for microscopic examination. Routinely the lung, liver, kidney, spleen, pancreas, and adrenal were examined, while in many cases other tissues such as the heart, muscle, bone marrow, testis, bladder, optic nerve, sciatic nerve, stomach, and intestines were also studied.

All of the exposed rats, except a few that died from other causes, survived 130–139 exposures over a period of 6 months and made good weight gains throughout the experimental period. Never-

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theless, these rats showed definite signs of eye and nose irritation during the exposures and often appeared somewhat dirty and unkempt.

Microscopic examination of tissues from the exposed and control groups revealed no essential differences between the two groups, although organ-weight records on the liver, kidney, spleen, and testis showed that in some cases there was a very slight increase in the weight of the liver and kidney in some of the exposed animals over that of the controls. These differences were so slight that their significance is quite doubtful.

The results of repeated blood examinations on 18 control rats and 18 rats receiving repeated 7-hour exposures to 6.0–6.3 mg./l. were strikingly similar for the two groups throughout an experimental period of 6 months, during which the experimental animals received 137–139 exposures. At no time did the total erythrocyte count, hemoglobin concentration, total leucocyte count, or differential count of the exposed group vary significantly from the values obtained on the control group.

About 10% of all the guinea pigs that were given repeated exposures to 6.0–6.3 mg./l. died after the first few exposures. Microscopic examination of the tissues from these guinea pigs revealed pronounced lung irritation, characterized by congestion, many hemorrhages, edema, exudation, and a general acute inflammatory reaction. No doubt these deaths resulted directly from this acute lung irritation. All of the guinea pigs that survived the first few exposures lived throughout an experimental period of 6 months during which they received 134–139 exposures, except a few animals whose death was due to other causes. However, the guinea pigs that survived gained weight very slowly, showed definite signs of eye and nose irritation, and often appeared dirty and unkempt.

Tissues from guinea pigs that had received 134–139 exposures showed no significant histopathological lesions other than a few healed pulmonary lesions. Organ-weight records on the liver, kidney, spleen, and testis revealed no essential differences between the exposed and control groups.

Of the 12 rabbits that received repeated exposures to 6.0–6.3 mg./l. one died from unknown causes after 45 exposures, while the others were sacrificed after 68, 140, 141, 141, 207, 208, 221, 226, 226, 252, and 264 exposures, respectively. All of the exposed rabbits made good weight gains, showed no evident reaction, and appeared to be in excellent condition throughout the experimental period. No significant pathological lesions were found in any of the exposed rabbits at the end of the experiment.

Repeated determinations of total erythrocyte count, hemoglobin concentration, total leucocyte count, and differential count on 4 control rabbits and on 3 rabbits receiving repeated exposures to 6.0–6.3 mg./l. throughout an experimental period of one year, revealed no significant differences in the blood picture of the two groups of rabbits.

Ophthalmoscopic examinations[1] failed to reveal any pathological changes in the eyes of rabbits that had received as many as 211–226 repeated exposures to 6.0–6.3 mg./l.

Two female monkeys survived 252–264 exposures over a period of 12 months, while 2 male monkeys survived 142 exposures during a 7-months period. Throughout this experiment these monkeys showed no evidence of irritation or intoxication and appeared to be in excellent condition. At the end of the experiment these 4 monkeys showed no gross or microscopic pathological lesions.

Repeated blood studies were made upon the 4 exposed monkeys and upon 3 control monkeys. No significant differences were found between the two groups.

An ophthalmoscopic examination of one of the female monkeys after the 210th exposure failed to reveal any pathological changes.

3.0 milligrams per liter (approx. 630 p.p.m.):

Since the previous work showed that guinea pigs were definitely affected by repeated exposures to 6.0–6.3 mg./l., it was necessary to give guinea pigs repeated exposures to a lower concentration.

Twenty-four guinea pigs were given 123–130 seven-hour exposures to 3.0 mg./l. over a period of 6 months. In order to have adequate control data, the 24 control guinea pigs were subjected to exactly the same routine as the exposed guinea pigs, even to being exposed regularly in an exposure chamber operated exactly like the styrene exposure chamber with the exception that no monomeric styrene was introduced into the air stream.

Throughout the experimental period of 6 months the animals in both groups appeared to be in good condition and made comparable weight gains. At the end of the experiment no gross or microscopic

[1] Examinations by M. H. Pike, M.D.

pathological lesions were found in any of the animals examined, and the weights of the liver, kidneys, spleen, and testis for the animals in the two groups were within the same range.

Oral Administration

Fifty-nine rats were given by stomach tube single oral doses of monomeric styrene dissolved in olive oil and emulsified in gum arabic solution. All of the animals that survived were observed for a period of 2 weeks or until it was certain that they had fully recovered. It was found that the 100% survival dose for rats was 1.6 g./kg. and the 100% lethal dose for rats was 3.0 g./kg.

Repeated oral doses of 2.0, 1.0, 0.5, and 0.1 g./kg. were given to rats, 5 days a week, over a period of 28 days unless death intervened.

A very few oral doses of 2.0 g./kg. (30% solution) produced a pronounced irritation of the esophagus and stomach which quickly resulted in the death of the animal. Even 1.0 g./kg. often caused a similar reaction after a few doses, with deaths in some cases before the end of the 28-day experimental period.

Although 5 male rats survived 20 oral doses of 0.5 g./kg., they made rather poor weight gains throughout the experimental period. At the end of the experiment no significant pathological lesions were found in these rats except a slight local reaction in the esophagus and stomach. Quite probably this local irritation resulted in a decreased food intake with consequent poor weight gain. Repeated blood examinations on these 5 male rats and upon 5 control male rats gave quite similar results in the two groups.

All rats survived 20 repeated oral doses of 0.1 g./kg. Throughout the experimental period they appeared to be in excellent condition and made good weight gains. At autopsy, no significant pathological lesions were found in these animals.

Skin Application

The effect of monomeric styrene upon the skin was tested on rabbits, using the method which has been described previously (2).

One application of the undiluted material to the ear of a rabbit caused no appreciable reaction, while 20 such applications over a period of 4 weeks produced moderate irritation with blistering and hair loss. Two applications bandaged onto the shaven abdomen produced marked irritation with some denaturation. This reaction produced by monomeric styrene is quite similar in type and intensity to that produced by other aromatic hydrocarbons such as toluene and benzene.

Metabolism Studies

Metabolism studies were carried out on female rats and rabbits that were receiving either repeated oral doses or repeated vapor exposures to monomeric styrene. During the experiments the animals were caged in individual metabolism cages; daily urine collections were made and daily records were kept of body weight, food and water consumption, and urine volume. In most cases an experimental period of 15 days was used. This period was divided into 3 parts: a 5 day control period, a 5-day period during which the animal was either fed monomeric styrene or exposed to its vapor, and a 5-day "follow-up" period. Usually the daily urine collections were made up into single 5-day composite samples for analytical purposes.

Routinely, the inorganic sulfate, total sulfate, total sulfur, total nitrogen, and "total benzoic acid" were determined on the composite urine samples.

The inorganic sulfate was determined by acidifying the urine, precipitating the sulfate with $BaCl_2$, filtering through a Gooch crucible and weighing the dried $BaSO_4$, essentially according to the method originally described by Folin (3). After acid hydrolysis of the urine, the same method was used to determine total sulfate. The total sulfur determination was carried out according to the method of Benedict (4) in which the urine is evaporated to dryness with Benedict's copper nitrate-potassium chlorate solution, ignited, the residue taken up in water, and the sulfate precipitated with $BaCl_2$.

In order to determine the "total benzoic acid" (more accurately designated as the "total titrational acidity of the ether extract of hydrolyzed urine") a slight modification of the method described by Quick (5) was used. The urine was made acid to Congo red with HCl, an aliquot hydrolyzed by refluxing with 20% HCl, and then thoroughly extracted with ether using a continuous extractor. This ether extract was evaporated to dryness, the residue taken up in toluene and titrated with 0.1 N sodium alcoholate using phenolphthalein as an indicator.

Total nitrogen was determined by the Kjeldahl method. Only those animals in which a constant N/S ratio was maintained are included in this report.

Monomeric styrene was administered in repeated

doses of 0.27, 0.54, and 0.81 c. c./kg. to rats and rabbits, and metabolism studies conducted according to the procedure described above.

Although the total urinary sulfur excretion remained practically constant or even decreased a little, there was an increase of 50 to 250% above the normal quantity of neutral sulfur excreted. This increase was the greatest in the animals receiving the highest doses and was greater in the rats than in the rabbits. Concomitant with this increase in neutral sulfur there was a decrease in inorganic sulfate excretion with practically no change in the excretion of ethereal sulfate. The per cent of the total sulfur excreted as neutral sulfur increased from a control value of 10 to 15% to values of 30 to 50%, the rats showing a slightly greater increase than the rabbits. In some cases there was a slight increase in the percent of the total sulfate excreted as ethereal sulfate; however, this increase was due to the decreased total sulfate excretion rather than to an appreciable increase in the quantity of ethereal sulfate excreted.

The "total benzoic acid" excretion increased two- to six-fold, the increase being the greatest in those animals receiving the highest doses. In isolation and identification studies on rabbit urine it was found that this "total benzoic acid" was actually benzoic acid and that it was excreted chiefly, if not entirely, as hippuric acid.

Calculations of the percent recovery of the orally administered monomeric styrene show that from 5 to 15% is accounted for by the increased neutral sulfur excretion while 50 to 90% is excreted as benzoic acid.

Rats and rabbits receiving repeated exposures to 0.0–6.3 mg./l. showed essentially the same changes that were found in the animals receiving repeated oral doses of monomeric styrene. In the rabbits the neutral sulfur excretion was doubled, thus accounting for 25% of the total urinary sulfur, while the "total benzoic acid" increased to 3 times the control value.

The neutral sulfur excretion in the exposed rats increased two- to four-fold, accounting for more than 50% of the entire urinary sulfur, while the "total benzoic acid" increased to 2–4 times the control value.

Summary and Discussion

The results of the experiments in which guinea pigs and rats were given single exposures to monomeric styrene vapor may be summarized as follows:

Reaction	Concentration of monomeric styrene	
	mg./l.	p.p.m. (vol.%)
First symptoms (eye and nose irritation)	3–6	650–1,300
Slight symptoms after several hours or maximum concentration for prolonged exposure (8 hours)	6–9	1,300–2,000
Maximum concentration tolerated for 60 minutes without serious systemic disturbances	12	2,500
Dangerous to life in from 30 to 60 minutes	46	10,000
Kills most animals in short time	A concentration nearer than that which can be attained at ordinary room conditions	

Most of the deaths which occurred during the single exposures were due to the primary action of monomeric styrene upon the central nervous system, while many of the delayed deaths resulted from the pneumonia which frequently developed following acute lung irritation.

It should be noted that since monomeric styrene has a relatively low vapor pressure at ordinary room temperatures, it is impossible to attain as high a vapor concentration of this material as can be obtained with many other substances of comparable vapor toxicity. Thus a concentration of approximately 10,000 p.p.m. monomeric styrene is the maximum concentration that can be obtained under ordinary room conditions.

From the acute vapor work on animals it is evident that the irritating properties of monomeric styrene are so marked it is unlikely man would tolerate a concentration of 6 mg./l. (1,300 p.p.m.) for more than a very few minutes, while concentrations above this are definitely prohibitive. From the practical standpoint, such a warning property affords a safeguard against acutely hazardous concentrations.

The results of repeated exposures of animals to monomeric styrene show that rabbits and monkeys can tolerate repeated exposures to 0.0–6.3 mg./l. (1,300 p.p.m.) for an experimental period of at least 6 months without exhibiting any signs of intoxication, while rats do equally well at this concentration except for evident eye and nose

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irritation. [illegible] affected by [illegible] they tolerate 3 studies on rats reveal any [illegible] repeated expos

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2. Adams, E. M Rowe, V. compounds dermatitis. Hyg. Sect.

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irritation. Although guinea pigs are definitely [illegible] repeated exposures to 6.0–6.3 mg./l., they tolerate 3.0 mg./l. very well. Frequent blood counts on rats, rabbits, and monkeys failed to reveal any changes in the blood picture during repeated exposures to 6.0–6.3 mg./l.

These experimental results indicate that repeated exposures to a concentration of approximately 3 mg./l. (650 p.p.m.) would probably produce no serious disturbances in man; however, since this concentration is definitely irritating to the eyes and nose of man, it probably would not be tolerated voluntarily for any great length of time except possibly in cases of habituation. A concentration of approximately 2 mg./l. (400 p.p.m.), which gives a disagreeable odor but does not produce appreciable eye or nose irritation in man, should present no serious industrial hazard and is suggested tentatively as the permissible limit for repeated exposures.

Tests upon rabbits show that monomeric styrene has an action on the skin quite similar to that of toluene and benzene, while industrial experience indicates that repeated exposures on the skin of man may produce a dermatitis. Apparently monomeric styrene presents essentially the same hazards of dermatitis as do the common aromatic hydrocarbons (toluene and benzene) and should be handled with the same precautions to avoid skin contact that are now observed with these solvents.

The metabolism studies on rats and rabbits show that monomeric styrene, when administered orally or inhaled, causes a marked rise in the "total benzoic acid" and the neutral sulfur excretion. Calculations of the percent of the orally administered styrene recovered in the urine indicate that 50 to 90% is excreted as benzoic acid and 5 to 15% as a neutral sulfur compound.

The results with animals indicate that an increased "total benzoic acid" and neutral sulfur excretion might be expected in man following exposure to monomeric styrene vapor.

Conclusions

Vapor concentrations of monomeric styrene which are acutely dangerous to guinea pigs and rats range from 10–12 mg./l. (approx. 2,400 p.p.m.) for an 8-hour exposure to 40 mg./l. (10,000 p.p.m.) for an exposure of 30–60 minutes. Higher concentrations are impossible to attain at ordinary temperatures. Immediate deaths result from the primary action upon the central nervous system, while many delayed deaths result from the pneumonia which frequently develops following acute lung irritation.

The extreme eye and nose irritation produced in man by a concentration of 6 mg./l. (1,300 p.p.m.) affords a definite safeguard against voluntary exposure to acutely hazardous concentrations.

Repeated 7 to 8-hour exposures to monomeric styrene vapor at a concentration of 6.0–6.3 mg./l. are tolerated without evidence of toxic reaction by rats, rabbits, and monkeys. No changes in the blood picture are observed in animals receiving such exposures. Repeated exposures to 3.0 mg./l. are well tolerated by guinea pigs.

A concentration of approximately 2 mg./l. (400 p.p.m.), which gives a disagreeable odor but does not produce appreciable eye or nose irritation in man, should present no serious industrial hazard and is suggested tentatively as the permissible limit for repeated exposures.

Monomeric styrene produces a reaction on the rabbits' skin which is very similar to that produced by the common aromatic hydrocarbons. In industrial use the same precautions should be observed to avoid skin contact as are now followed in the handling of other aromatic hydrocarbon solvents.

Monomeric styrene, when administered orally or inhaled as a vapor, causes a marked rise in "total benzoic acid" and neutral sulfur excretion in rats and rabbits. Similar changes might be expected to occur in man.

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TRANSCRIPT OF PROCEEDINGS

IN THE SUPERIOR COURT

OF THE STATE OF DELAWARE

IN AND FOR NEW CASTLE COUNTY

MONSANTO COMPANY, Plaintiff,	:	Civil Action Number
v.	:	88C-JA-118-1-CV
AETNA CASUALTY & SURETY COMPANY, et al., Defendants.	:	NON-ARBITRATION CASE

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