Evaluation of Telemetry in Determining Toxicity of Aerosol Preparations

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Abstract □ The effect of selected aerosol products and fluorinated hydrocarbon propellants on the heart rate and respiration of rats was determined in whole body exposure chambers by biotelemetry. Electrocardiogram and respiratory transmitters were used to broadcast selected FM signals to a nearby receiver and physiological recorder for visual display. Each rat was subjected to a single 360-min exposure of the aerosol product or propellant, kept under observation for toxicological responses, and then examined for histopathological signs of toxicity. The fluoroalkane propellants used depressed the heart rate in high concentrations. First degree atrioventricular block and T wave inversion were also observed in some animals. The propellants tested also had a depressant effect on respiratory rate and minute volume. The selected aerosol product produced similar effects but at concentrations much higher than would be experienced during normal consumer use. Histopathological examination revealed greater tissue damage with the aerosol product than with the propellants alone. The cardiac and respiratory abnormalities occurred only after exposure to high concentrations of the fluoroalkane propellants. Also, the pathology report gave no indication of the existence of "thesaurosis" or pulmonary granulomatosis.

Keyphrases □ Telemetry—evaluation as technique for determining toxicity of aerosol preparations □ Toxicity, aerosols—evaluation of telemetry as technique for determination □ Aerosols—evaluation of telemetry for determining toxicity □ Propellants, aerosols—evaluation of telemetry for determining toxicity □ Fluorinated hydrocarbons—use of telemetry in evaluating toxicity of aerosol preparations

In the last decade there has been a significant increase in the marketing and use of aerosol products for medicinal, cosmetic, and household purposes. This has led to the occasional questioning, by both consumer and professional organizations, of the safety of the various aerosol preparations commercially available. Recently, charges of cardiac and pulmonary toxicity have been leveled at certain commercial aerosol preparations. The ability of any particular product to produce injury, and the risk or probability that the injury will occur, must be carefully considered in the toxicological evaluation of commercial aerosols.

Bass (1), for example, reported several cases of sudden "sniffing death" from aerosol abuse, while Taylor and Harris (2) demonstrated that asphyxia could lead to cardiac arrhythmias in animals following the inhalation of some aerosol propellants. Similar toxic effects were also reported for some cosmetic aerosols. Flowers and Horan (3) reported arrhythmias in dogs following inhalation of fluoroalkane propellants despite careful maintenance of normal arterial oxygen tension and pH. Kilen and Harris (4) reported a direct depressant effect of dichlorodifluoromethane on the contractility *in vitro* of the isolated rat papillary muscle.

In addition, Reinhardt *et al.* (5) reported that the cardiac sensitization was a possible mechanism for the aerosol sniffing deaths. In subsequent experiments, Taylor *et al.* (6) proposed that either sensitization of the myocardium to catecholamines or a direct depressant action on the myocardium might explain the cardiotoxicity of the fluorocarbon propellants.

Table I—Particle-Size Distribution of Antiperspirant Preparation

Diameter of Particle, μm	Mass of Collected Material, g	Cumulative Mass, g	Cumulative Weight, %
1	0.0416	0.0024	1.895
2	0.0254	0.0088	6.932
4	0.0327	0.0269	21.309
8	0.0182	0.0597	47.134
16	0.0064	0.0851	67.156
32	0.0024	0.1267	100.00

It is well known that the deposition of aerosols in the respiratory tract is a function of several variables (7–10): particle size and shape, density of particles, hygroscopicity, anatomical pattern of the respiratory tract of the test animal, and pathological changes in respiratory patterns (11). All of these variables must be considered when evaluating a possible test system or toxicological data concerning a particular aerosol product.

Bergman *et al.* (12) first indicated the possibility of a connection between the use of aerosols and granulomatous lesions in the lungs; their findings were later supported by others (13–15). Ward (16) indicated that certain aerosol deodorants might be the cause of pulmonary lesions in susceptible users.

Other workers (17–20), however, failed to produce any of these pulmonary lesions, thesaurosis, or granulomatosis in experimental animals. Many of the variables between experimental animals and humans could explain these discrepancies. There are obvious differences in the anatomy and physiology of the respiratory systems of small animals and humans (15). In general, the dimensions of the upper respiratory structures decrease and the tortuosity increases as the size of the animal decreases. The ratio of surface area to volume of the upper respiratory tract also increases. Therefore, the inherent efficiency of the upper respiratory tract in trapping small particles increases as the animal becomes smaller.

Hatch and Gross (21) stated that the pulmonary air spaces are not remarkably smaller in small animals *versus* humans. The lesser lung volume in small animals is accounted for by fewer, rather than smaller, alveoli. However, because of the higher respiratory efficiency, the fraction of inhaled particles deposited in the pulmonary air spaces is lower in small animals for particles greater than 1 μm in diameter (22).

Table II—Mean Heart Rate and Mean Percent Change in Heart Rate of Rats following 360 min of Aerosol Exposure

Treatment[a]	Mean Heart Rate[b]	p Value[c]	Mean Percent Change in Heart Rate[d]	p Value
Dichlorodifluoromethane[e]	389.10 ± 23.50	0.02	−9.71 ± 5.28	0.001
Dichlorodifluoromethane–trichloromonofluoromethane[f]	399.91 ± 25.92	0.001	−7.99 ± 6.08	0.02
Antiperspirant[g]	384.00 ± 31.32	0.02	−7.54 ± 7.61	0.05
Control I	416.46 ± 6.75	—	−0.30 ± 1.62	—
Control II	444.28 ± 6.44	—	−0.96 ± 1.45	—

[a] Each group was composed of five animals. [b] Beats per minute ± standard deviation. [c] For significance, $p < 0.05$. [d] Percent change ± standard deviation. [e] Compared with Control I. [f] Compared with Control II. [g] Compared with Control I.

In humans, particles between 0.5 and 5.0 μm in diameter are the optimum size for deposition in the lung (7). As additional evidence, it would be desirable to reproduce the reported human lesions in laboratory animals; failure to do so, however, does not constitute proof that the material is harmless to humans (14).

Because of the apparent drawbacks in methodology used by these and other investigators to record cardiovascular and respiratory function during aerosol exposure, the present investigation was undertaken to evaluate the potential of a new technique, telemetry, as a means for recording vital signs during the exposure of laboratory animals to propellants and/or specific aerosol products. It was hypothesized that telemetry could have possible advantages over the more common exposure techniques available and that experimentation was necessary to prove or disprove these possible advantages.

EXPERIMENTAL

The aerosol preparations used were prepared by the cold filling process (23). The antiperspirant preparation used was prepared according to the following formula (24) (percent w/w): aluminum chlorhydroxide[1], 35.0; hexachlorophene[2], 2.0; isopropyl myristate, 56.0; fumed silica[3], 6.0; and butylated hydroxytoluene, 1.0.

The aerosol consisted of 10% by weight of concentrate and 90% by weight of dichlorodifluoromethane[4]–trichloromonofluoromethane[5] (50:50). Before pressurizing, a magnetic stirring bar was placed in the container to facilitate mixing. The particle-size distribution of this preparation was determined using an impaction technique (25, 26)[6]. The spray rate was determined by a weight-by-difference method.

The exposure apparatus consisted of a 30.48 × 30.48 × 30.48 cm (1 cu ft) chamber. The air flow through the chamber was adjusted to approximately 2.5 liters/min. An automatic aerosol delivery device (27) mounted on a magnetic stirrer served both to activate and to mix the aerosol preparations.

Male Sprague–Dawley rats (200–300 g) were trained to wear a 2.54-cm wide elastic harness (28), containing either an electrocardiogram (ECG) or respiratory transmitter. In addition to the preconditioning, a period of adjustment was required to allow the animals to adapt to the transmitter, harness, and chamber. Sixty minutes was found to be appropriate for this purpose.

The animals were exposed individually to the various test agents. The automatic aerosol delivery apparatus was adjusted to deliver a 7.5-sec spray every 10 min for 6 hr (18, 29). The animals were exposed to three preparations: an antiperspirant preparation, dichlorodifluoromethane, and a blend of dichlorodifluoromethane–trichloromonofluoromethane (50:50). During the exposure, either ECG or respiratory recordings were made. The animals were observed for weight changes and other toxicological manifestations for 7 days; they were then sacrificed and selected tissues were excised for histopathological examination.

An additional group of male Sprague–Dawley rats was exposed individually three times a day to a 15-sec spray of the aerosol antiperspirant for 30 days. The animals were exposed singly and allowed to remain in a sealed chamber for 15 min following each spray (17, 30). These animals were observed for weight changes.

Analysis of the fluoroalkane propellant concentration in the chamber was determined by GC[7], using a thermal conductivity cell. The injection port temperature was set at 140°, the detector temperature was 158°, the filament current was 200 mamp, and the attenuator was set at 2×. Helium was used as the carrier gas at a flow of 40 ml/min. Two glass columns were used. The first was 2.4 m × 0.3 cm (8 ft × 0.125 in.), packed with 10% polyethylene glycol 20M on 80–100-mesh Aerograph A-3-55. The second was 1.8 m (6 ft) of 80–100-mesh Poropak Q. The columns were operated at a temperature of 120°. A strip-chart recorder was used at a chart speed of 1.7 cm (0.66 in.)/min.

RESULTS AND DISCUSSION

GC analysis yielded a chamber concentration of 304.54 mg/liter for the propellant blend of dichlorodifluoromethane–trichloromonofluoromethane (50:50), 382.51 mg/liter for dichlorodifluoromethane, and 219.69 mg/liter for the propellant contained in the antiperspirant preparation.

The particle-size distribution of the antiperspirant preparation is shown in Table I. Based upon the cumulative weight percent, the mass median diameter of the particles is approximately 8 μm. Approximately 21% of the particles are 4 μm in diameter or smaller and, therefore, are in the respirable range (7).

The effects of impingement of particulate matter on the fur of experimental animals should be considered in every inhalation experiment; it probably explains the excessive lowering of concentration that occurs when the volume of animal used is in excess of 5% of the chamber volume (31). Calandra and Kay (17), for example, reported that experimental animals weighing 200–300 g occupy a volume between 1 and 2 liters. Thus, to keep the ratio of chamber to animal volume as large as possible, the rats were exposed individually in this study.

A temperature range of 21–23° and a relative humidity of approximately 50% have been suggested as suitable exposure conditions for most animals; rats and mice thrive at somewhat higher temperatures (32). Thus, the conditions of this study adhered as closely to these guidelines as possible.

The effects of the fluoroalkane propellants on heart rate are shown in Table II. The dichlorodifluoromethane and the antiperspirant groups were compared with Control I, and the rats exposed to the dichlorodifluoromethane–trichloromonofluoromethane blend were compared with Control II. All treatment groups exhibited a significant percent decrease in heart rate. When compared with the antiperspirant group, dichlorodifluoromethane and dichlorodifluoromethane–trichloromonofluoromethane did not significantly decrease the heart rate on a percent basis (p values of <0.6 and <0.9, respectively).

[1] Chlorhydrol, Impalable, Reheis Chemical Co., Berkeley Heights, N.J.
[2] Since the time this study was initiated, the Food and Drug Administration has placed restrictions on the use of this compound.
[3] Cab-O-Sil M-5, Cabot Corp., Boston, Mass.
[4] Propellant 12.
[5] Propellant 11.
[6] Cascade Impactor, model CI-S-6, Scientific Advances, Inc., Columbus, Ohio.
[7] Aerograph model 350 B chromatograph.

Table III—Mean Percent Change in Respiratory Rate and Minute Volume of Rats following 360 min of Aerosol Exposure

Treatment[a]	Mean Percent Change in Respiratory Rate[b]	p Value[c]	Mean Percent Change in Minute Volume[b]	p Value[c]
Dichlorodifluoromethane	−3.57 ± 3.68	0.5	−3.52 ± 3.69	0.4
Dichlorodifluoromethane–trichloromonofluoromethane	−7.42 ± 4.08	0.05	−7.29 ± 4.13	0.02
Antiperspirant	−4.11 ± 2.89	0.3	−4.09 ± 2.90	0.2
Control	−1.99 ± 3.52	—	−1.58 ± 3.67	—

[a] Each group was composed of five animals. [b] Mean percent change ± standard deviation. [c] For significance, $p < 0.05$.

Table IV—Effects of Aerosol Propellants and a Selected Aerosol Product on 7-Day Weights of Rats following a Single 360-min Aerosol Exposure

Treatment[a]	Mean Weight[b]	p Value	Mean Percent Daily Weight Gain[b]	p Value[c]	Mean Percent Change[b]	p Value[c]
Dichlorodifluoromethane	263.07 ± 22.50	0.7	3.5 ± 1.9	0.7	13.4 ± 9.7	0.7
Dichlorodifluoromethane–trichloromonofluoromethane	258.74 ± 22.75	0.4	3.5 ± 1.7	0.6	12.9 ± 9.9	0.8
Antiperspirant	259.80 ± 8.51	0.3	−1.5 ± 2.5	0.01	−4.1 ± 3.1	0.001
Control	269.07 ± 19.96	—	3.0 ± 1.6	—	11.2 ± 8.2	—

[a] Each group was composed of five animals. [b] Mean ± standard deviation. [c] For significance, $p < 0.05$.

The fluoroalkane propellants produced significant changes in heart rate in concentrations of approximately 200, 300, and 400 mg/liter, with the 50:50 combination of dichlorodifluoromethane–trichloromonofluoromethane producing the most significant decrease in heart rate ($p < 0.001$).

The control heart rates were shown to vary slightly, as might be expected (28) when measuring heart rate for prolonged time intervals. Mean decreases in heart rate of 0.30 ± 1.62 and 0.96 ± 1.45% were shown for Controls I and II, respectively, after 360 min in the exposure chamber. The rats exposed to dichlorodifluoromethane showed a mean decrease in heart rate of 9.71 ± 5.28%, while those exposed to the propellant blend and the antiperspirant preparation showed mean decreases in heart rate of 7.99 ± 6.08 and 7.54 ± 7.61%, respectively.

The animals exposed to the antiperspirant preparation showed the greatest overall depression in heart rate, 18.02%. The group exposed to dichlorodifluoromethane exhibited a net decrease in heart rate of 11.04%, while those exposed to the propellant blend exhibited a net decrease of 16.21%. The toxicity of fluoroalkane propellants has been reported to vary inversely with the strength of the carbon–fluorine bond; the strength of the bond also varies with the number of fluorine atoms in the molecule (33).

In accordance with these findings, trichloromonofluoromethane was reported to be more toxic than dichlorodifluoromethane (34). The present study reflects this concept in that addition of trichloromonofluoromethane to dichlorodifluoromethane amplified the myocardial depression. However, neither pure propellant nor the propellant blend produced a significant decrease in heart rate ($p < 0.4$); the percent decrease in heart rate was also not significantly different ($p < 0.6$).

High concentrations of fluoroalkane propellants have been reported to produce anesthesia, narcosis, cardiac arrhythmias, cardiac sensitization, and death (5, 34–36). Bradycardia, cardiac arrhythmias typical of first degree A-V block, inversion of the T wave, loss of the P wave, lengthening of the QRS interval, and death were reported (37) as results of exposure to dichlorodifluoromethane–trichloromonofluoromethane (50:50) in concentrations of 200 and 400 mg/liter. Arrhythmias, bradycardia, and T wave depression were also reported by other investigators (2, 3, 6).

The present study agrees with these reports of bradycardia produced by the propellants, either alone or in combination. The bradycardia observed was consistent with the direct depressant effect of dichlorodifluoromethane on myocardial contractility reported by Kilen and Harris (4). The present study showed transient arrhythmias, which mainly occurred between 300 and 360 min of exposure. The observed increase in the P-R interval was likely a reflection of first degree A-V block (38). As the bradycardia continued, the QRS interval was increased over normal values. The P wave, which is recorded as soon as the impulse leaves the S-A node (39), disappeared, indicating diminution of atrial activation, such as S-A block or atrial fibrillation (40). An inverted T wave, which was only seen sporadically in response to the propellant blend, indicates abnormal conduction through the A-V node (39).

All rats in the treated groups exhibited ataxia and hypomotility after approximately 200 min of aerosol exposure. Only animals exposed to the propellant blend showed tremors, salivation, and a kyphotic position. Recovery always occurred within 5 min after removal from the exposure chamber, and no deaths were recorded during either the 360-min exposure or the 7-day observation that followed.

The effects of inhalation of either propellants or the antiperspirant formula on respiration are shown in Table III. Of the various treatments used, only the propellant blend produced significant changes in the rate and volume of respiration. As with heart rate, the control respiratory rate was shown to have a slight variation throughout the exposure period; the net decrease in respiratory rate was 1.99 ± 3.52%.

The propellant blend alone produced a significant decrease in respiratory minute volume ($p < 0.02$). The present study demonstrated a concentration-dependent decline in respiratory rate with the propellant blend, since 300 mg/liter but not 200 mg/liter produced a significant depression; no significant effects were produced by 400 mg/liter of dichlorodifluoromethane.

The effects of inhalation of the various aerosols on weight gain are shown in Tables IV and V. It is well known that weight loss can serve as an index of toxicity (41, 42). Only the antiperspirant preparation produced any significant weight loss ($p < 0.001$) or de-

Table V—Mean Starting and Ending Weights of Rats following 30 Days of Three Times a Day Aerosol Exposure

Treatment[a]	Mean Starting Weight[b]	p Value[c]	Mean Ending Weight[b]	p Value[c]
Antiperspirant	243.9 ± 10.5	0.5	299.6 ± 40.2	0.001
Control	249.0 ± 15.2	—	440.1 ± 55.1	

[a] Each group was composed of eight animals. [b] In grams ± standard deviation. [c] For significance, $p < 0.05$.

Table VI—Histopathological Examination of Selected Rat Tissue following Aerosol Exposure[a]

Pathological Diagnosis	Dichlorodifluoromethane[b]	Dichlorodifluoromethane–Trichloromonofluoromethane[b]	Antiperspirant[b]	Control[b]
Passive pulmonary congestion	0/5	0/5	0/5	2/5
Nonspecific hepatitis	0/5	0/5	0/5	1/5
Toxic hepatitis (possible)	0/5	0/5	4/5	0/5
Acute reactive splenitis	0/5	0/5	3/5	0/5
Interstitial nephritis	0/5	0/5	1/5	0/5
Acute liver congestion	0/5	0/5	1/5	0/5
Interstitial edema of the heart	0/5	0/5	1/5	0/5
Acute pulmonary congestion	0/5	0/5	2/5	0/5
Nonspecific pneumonitis	4/5	3/5	2/5	1/5
Bronchopneumonia	1/5	0/5	0/5	1/5

[a] Examinations performed by G. Forman, Director of Pathology, Central Health Laboratories, Wantagh, N.Y. [b] Number responding/number tested.

crease in weight gain ($p < 0.001$). Because the propellants themselves did not produce depression of weight gain, they cannot be implicated in the changes produced by the antiperspirant preparation. It follows, then, that particulate matter may produce a toxic sequela. The animals exposed to dichlorodifluoromethane and dichlorodifluoromethane–trichloromonofluoromethane exhibited a greater increase in weight gain than did the control group. This finding is consistent with the reported (42) weight gain increases in animals treated with fluoroalkane propellants.

The results of the histopathological examination of selected tissues are summarized in Table VI. In the opinion of many investigators (43, 44), there is no animal of choice for inhalation experiments. Small rodents are known, for instance, to be subject to respiratory infections, yet they are often used in inhalation experiments (2, 17). In this study, using rats, it was shown that the antiperspirant preparation produced the greatest histopathological changes. Some responses were toxic hepatitis, acute reactive splenitis, interstitial nephritis, acute congestion of liver, interstitial edema of the heart, acute pulmonary congestion, and nonspecific pneumonitis; no pulmonary granulomatosis was observed.

The animals exposed to the fluoroalkane propellants showed only a nonspecific pneumonitis; bronchopneumonia was reported in one rat exposed to dichlorodifluoromethane. The control group showed similar lung pathology; thus no significant pathology was found in rats exposed to the fluoroalkane propellants. This would appear to suggest that the propellants themselves do not produce pulmonary and systemic pathology in rats exposed to commercial aerosols, as has been suggested for other preparations (15).

CONCLUSIONS

The use of telemetry facilitates the measurement of heart and respiratory rates in the unrestrained animal. Furthermore, there are no trailing wires and the animals do not have to be removed from the test atmosphere for recordings to be made, as is often the case when vital functions are recorded in other ways. The latter advantage is very important since the test animals invariably recover from any visible effects of the aerosol product almost immediately when removed from the exposure chamber. Although a period of adjustment is necessary before the animals will easily accept the transmitter pack, once adaptation to the presence of the pack does occur, the only limitation to the length of continuous telemetric recording is the life of the batteries powering the transmitter.

These studies should in no way be construed to imply that aerosol products used by the consumer will produce similar toxic effects. The doses and techniques used were selected to produce toxicity so the value of telemetric monitoring could be evaluated. Thus, the doses and exposure periods are considerably higher than would likely be experienced by a consumer of any commercial aerosol.

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Mass Spectral Analysis of Medicinal Pyrazolidinediones

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Abstract □ For the facilitation of mass spectral analysis of medicinal pyrazolidinediones, fragmentation pathways of phenylbutazone, oxyphenbutazone, and sulfinpyrazone were established by means of deuterium labeling, metastable peaks, and accurate mass determinations. The major pathways are the McLafferty rearrangement of the molecular ion and formation of azobenzene and substituted azobenzene ions. The mass spectra of 1,2-diphenyl-3,5-pyrazolidinedione and the 4-methyl and 4,4-dimethyl derivatives are also discussed.

Keyphrases □ Pyrazolidinediones, medicinal—mass spectra, fragmentation pathways, mechanisms □ Phenylbutazone—mass spectrum, fragmentation pathways, mechanisms □ Oxyphenbutazone—mass spectrum, fragmentation pathways, mechanisms □ Sulfinpyrazone—mass spectrum, fragmentation pathways, mechanisms □ Mass spectroscopy—medicinal pyrazolidinediones, fragmentation pathways

Although medicinal pyrazolidinediones such as phenylbutazone have received wide attention in the past 20 years, only a few reports of their mass spectra have appeared recently (1–3). This technique was used for the characterization of degradation products of phenylbutazone (1, 2), but no details of the fragmentation pathways of phenylbutazone or the degradation products were described.

The fragmentation patterns of phenylbutazone, oxyphenbutazone, and their *O*-methyl and C-methyl derivatives were described (3), but that investigation did not include verification by evidence of metastable ions, accurate mass determinations, or use of labeled derivatives.

This paper presents the mass fragmentation patterns of 1,2-diphenyl-3,5-pyrazolidinediones of medicinal importance: phenylbutazone, oxyphenbutazone, and sulfinpyrazone, as well as some simpler model derivatives. The establishment of characteristic mass spectral patterns and fragmentation schemes for these compounds may facilitate further characterization of pharmaceutical degradation products, metabolic studies, and forensic analysis of this important class of medicinals.

EXPERIMENTAL[1]

All mass spectra were recorded on a mass spectrometer[2] at an ionizing potential of 70 ev. The samples were introduced *via* the direct probe and were vaporized at temperatures between 150 and 200°. Accurate mass measurements were made by the peak matching technique.

Phenylbutazone[3] (I), Oxyphenbutazone[3] (II), and Sulfinpyrazone[3] (III)—Compounds I–III had literature melting points and gave NMR and IR spectra consistent with their structures. Official assays gave values in excess of 98% for each compound. They were used without further purification.

[1] IR spectra were taken on a Unicam SP 1000 IR spectrophotometer, and NMR spectra were recorded on a Varian A-60D spectrophotometer using tetramethylsilane as the internal standard. UV spectra were obtained on a Unicam SP 1800 UV spectrophotometer. Elemental analyses were determined by Mr. W. Dylke.

[2] AEI MS-9.

[3] Gift of Geigy Pharmaceuticals.