# Comparison of Analgesic Effects of Isosteric Variations of Salicylic Acid and Aspirin (Acetylsalicylic Acid)

LEON THOMPKINS * and K. H. LEE ˣ

**Abstract** □ A reliable and sensitive method was used to compare the analgesic activities of salicylic acid and aspirin (acetylsalicylic acid) and several phenoxy substituted isosteric pairs. Those isosteric compounds studied did not show analgesic activity. The analgesic activity of aspirin was more than twofold greater than that of salicylic acid.

**Keyphrases** □ Aspirin—analgesic effects of isosteric variations, compared to salicylic acid isosteres □ Salicylic acid—analgesic effects of isosteric variations, compared to aspirin isosteres □ Analgesics—comparison of effects of isosteric variations of salicylic acid and aspirin □ Salicylates—comparison of analgesic effects of isosteric variations of salicylic acid and aspirin

Of the three major pharmacological actions of salicylic acid and aspirin (acetylsalicylic acid), their quantitative difference is most apparent in their analgesic effect. This difference in potency in experimental animals and in humans has been observed by several workers (1–5) and has been primarily responsible for the suggestion that aspirin's activity is not due to conversion to salicylic acid (6–8).

Although this suggestion was first made more than 50 years ago, there have been no attempts to resolve the question of whether aspirin is analgesic in its own right by using comparative analgesic studies of isosteric structural modifications. This seems especially significant in that isosteric substitution of $-CH_2-$ or —NH— for the phenoxy oxygen in aspirin would result in compounds stable or less susceptible to hydrolysis, which has been a major problem in assessing the relative effects of aspirin and salicylic acid on the biological system. Such studies might provide indications of the structural requirements for analgesic activity and information concerning the mechanism by which the salicylates exert their pharmacological effects.

Perhaps one reason that such studies were not carried out was the lack of reliable bioassay procedures for testing nonnarcotic analgesics. Aspirin and salicylic acid show activity as antinociceptive agents in many tests (9). However, most of them require high doses of salicylate to produce a minimal effect, are not clearly definitive, or are unreliable.

Guzman *et al.* (3) introduced a method that employs intraarterial injections of bradykinin in dogs, producing vocalization and other autonomic and behavioral reactions, to assess the activity of nonnarcotic and narcotic analgesics. In animals that respond, this method is superior to other analgesic tests if one considers overall reliability, sensitivity, and definitiveness. This method was later adapted to rats, and similar results were found (10). However, while Guzman *et al.* (3) used primarily the vocalization as the index to the pain response after the bradykinin injections in dogs, the latter workers described struggling, dextrorotation of the head, and occasional vocalization as indexes.

The effects of intraarterial injections of bradykinin into rats were used in this study to assess the analgesic activities of several phenoxy-substituted isosteric pairs of salicylic and acetylsalicylic acids. The analgesic effects of the following pairs were studied: acetylsalicylic acid (I) and salicylic acid (II), *N*-acetylanthranilic acid (III) and anthranilic acid (IV), acetylthiosalicylic acid (V) and thiosalicylic acid (VI), and 2-acetonylbenzoic acid (VII) and 2-methylbenzoic acid (VIII).

## EXPERIMENTAL

**Reagents**—The following were used: thiosalicylic acid[1] (mp 163–165°), *N*-acetylanthranilic acid[1] (mp 184.5–187°), acetylsalicylic acid[2] (ACS reagent, spectrophotometrically pure), homophthalic acid anhydride[2] (mp 139–141°), salicylic acid[3], chloroform[4] (technical), anthranilic acid[5], pyridine[6] (reagent grade), 2-methylbenzoic (*o*-toluic) acid[7], and sterile sodium chloride injection[8] USP (0.0009 g/ml). All other chemicals were reagent grade.

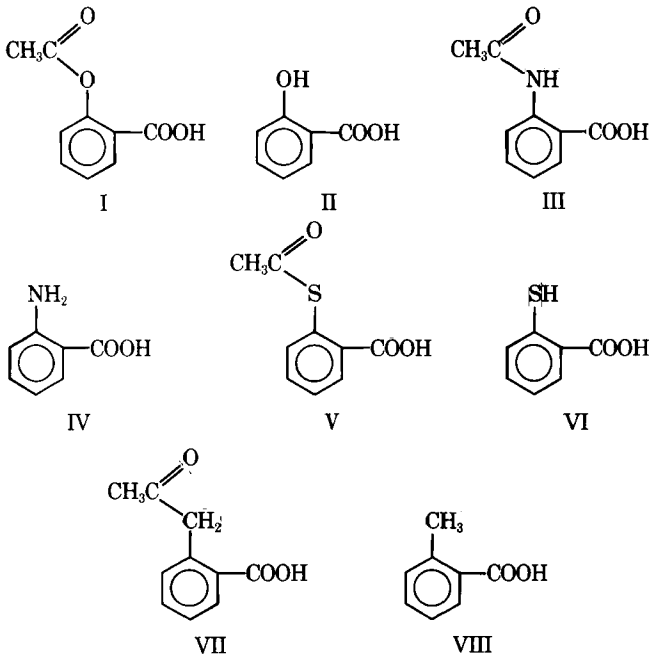
**Purification Procedures**—Thiosalicylic acid was recrystallized from ethanol and water as described in the literature (11). Pyridine was dried, distilled, and stored over sodium hydroxide pellets (12). Chloroform was purified, dried, and distilled (13). The distillate was used immediately.

**Synthesis of Acetylthiosalicylic Acid**—This compound was prepared using the method of Hinsberg (14). The crude product was dried over phosphorus pentoxide at room temperature under vacuum. The dried product was recrystallized several times from benzene until needles melting at 125° were obtained.

**Synthesis of 2-Acetonylbenzoic Acid**—This compound was prepared using the methods described by Schnekenburger (15, 16). These methods involve acetylation of homophthalic acid anhydride to obtain the 4-acetylhomophthalic acid anhydride, followed by alkaline hydrolysis with simultaneous decarboxylation of the product to 2-acetonylbenzoic acid (15). Colorless needles with the melting point of 2-acetonylbenzoic acid (122°), as described by Schnekenburger (16), were obtained.

**Restraining Cage**—For the measurement of analgesic activity, a restraining cage was designed in which the reactions of the animal to intraarterial injections of bradykinin could be observed and intraperitoneal injections of the test drug could be made (Fig. 1). The overall length of this cage was 20.3 cm (8 in.), and the distance between vertical supports was 7.6 cm (3 in.). The openings in the

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] City Chemical Corp., New York, N.Y.
[3] Baker analyzed reagent, J. T. Baker Chemical Co., Phillipsburg, N.J.
[4] J. T. Baker Chemical Co., Phillipsburg, N.J.
[5] Baker grade, J. T. Baker Chemical Co., Phillipsburg, N.J.
[6] Eastman Organic Chemicals, Distillation Products Industries, Rochester, N.Y.
[7] Eastman grade, Eastman Organic Chemicals, Distillation Products Industries, Rochester, N.Y.
[8] Provided by the University of California Hospital Pharmacy, San Francisco, Calif.




front and rear supports were holes with a depth of 3.7 cm (1.47 in.) and a width of 4.4 cm (1.75 in.). The top was removable; once the animal was positioned, the top was secured with wingnuts to hold the rat firmly but comfortably (Fig. 2). This cage was found to be suitable for these experiments.

**Measurement of Analgesic Activity**—Male Sprague–Dawley or Wistar rats were used in all experiments. A modification of the procedure of Deffenu *et al.* (10) was used in the testing of the analgesic activity of the various isosteric pairs. The rats, 230–320 g, were anesthetized with ether in an open mask and the right common carotid artery was catheterized, using a catheter consisting of two fused lengths of heparinized polyethylene tubing with inside diameters of 0.58 (PE 50) and 0.28 (PE 10) mm.

The catheter was inserted centripetally, passing through the subcutaneous tissues so that the portion of the tubing with the smaller diameter was inserted into the artery while that with the larger diameter protruded from the top of the head. After the tubing was anchored and the incision was closed, the tubing was opened to check blood flow, filled with heparin (40 units/ml in sterile saline injection USP), shortened so that the rat could not reach it, and plugged with a metal plug. At least 24 hr was allowed for each rat to recover prior to testing.

Rats were placed in the cages, and polyethylene 50 tubing extensions were attached to the projecting catheter *via* a hollow metal tube prepared from a length of No. 23 hypodermic needle. The tubing was taped to the top of the cage so that the animal would be unaware of the experimenter's handling of the distant end. Then 0.2 ml of normal saline was injected very slowly from the carotid artery, and no reaction of the animal was observed. This was followed by an injection of 0.5 $\mu$g of bradykinin in 0.2 ml of normal saline to determine if the animal was responsive to bradykinin. In about 99% of the rats, vigorous struggling and dextrorotation of the head were observed. Rats that were unresponsive to this dose of bradykinin were not used further. The sodium salt of the drug to be tested was injected intraperitoneally in normal saline. The volume of the injection was 0.25 ml/100 g or the smallest volume that could completely dissolve the salt.

Ten minutes after the injection of the drug, the animals were challenged with another 0.5-$\mu$g dose of bradykinin, preceded by a normal saline injection, and the animals' responses were carefully watched. The same procedure was repeated at 10-min intervals until the bradykinin effect reappeared.

If no effect was seen with a dose level of 1000 mg/kg for a particular drug, intraarterial studies with a dose level of 100 mg/kg were made. The intraarterial injections of the drugs were made in normal saline (0.5 ml/rat) *via* the same catheter used for the bradykinin injection. Otherwise, the procedure was the same as for the intraperitoneal injections.

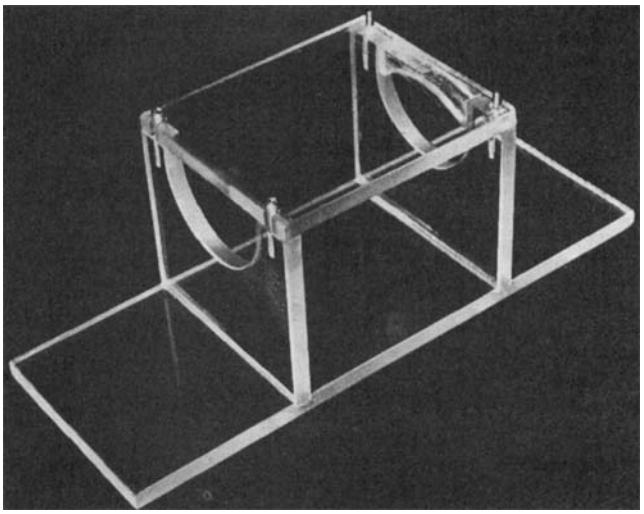


**Figure 1**—*Rat cage.*

The criterion chosen for evaluating protection was the disappearance of the bradykinin effect after two consecutive doses of bradykinin. The doses of drugs that showed no protection after five consecutive doses of bradykinin were assumed to be nonanalgesic in these studies. Each rat received one drug at one dose level, and five rats were used for each dose level. If a drug was found to be toxic, the $LD_{50}$ was determined.

The $ED_{50}$ and $LD_{50}$ values were obtained using Thompson's method (17) of interpolation between moving averages.

## RESULTS AND DISCUSSION

A summary of the data obtained in these experiments is contained in Table I, including the responses of the groups of rats to various doses of the drugs tested. Salicylic and acetylsalicylic acids showed clearcut analgesic activity *versus* bradykinin injections, intraperitoneally. Acetylsalicylic acid showed analgesic activity at a much lower dose level when intraarterial injections of the drug were made.

It is difficult to interpret the results obtained with *N*-acetylanthranilic and anthranilic acids. Both of the drugs seemed to afford protection *versus* bradykinin injections at lower dose levels intraperitoneally. However, at higher intraperitoneal dose levels and at a dose level of 100 mg/kg intraarterially, no analgesic activity was demonstrated. No other analgesic drugs demonstrating such anomalous behavior have been described. It is assumed that these two compounds have no analgesic activity as indicated in Table II, where the $ED_{50}$ values for compounds showing analgesic activity and the $LD_{50}$ values for compounds found to be toxic are reported.

Both Tables I and II show the toxicity of acetylthiosalicylic and thiosalicylic acids, the nonacetylated compound being more toxic. This toxicity was characterized by severe tremors and hyperventilation followed by a violent struggle just before death. All animals thus affected died within 50 min, and *rigor mortis* set in rapidly.

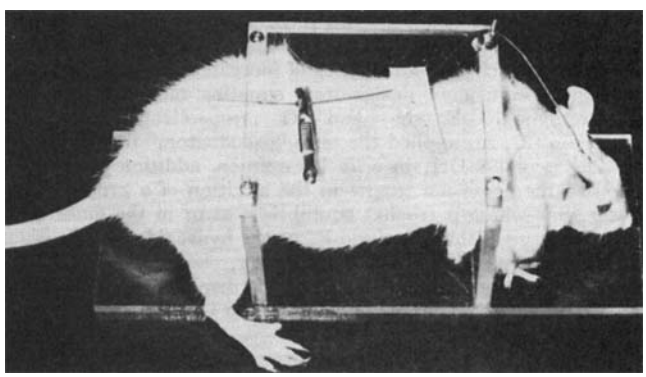


**Figure 2**—*Rat cage with rat.*

**Table I**—Effects of the Injection of Various Salicylate Isosteres *versus* Intraarterial Injections of Bradykinin in Rats

| Drug | Number of Rats Tested | Dose of Drug, mg/kg | Route[a] | Number Protected *versus* Bradykinin | Number of Deaths |
|---|---|---|---|---|---|
| Acetylsalicylic acid | 5 | 31.6 | ip | 1 | 0 |
| | 5 | 46.4 | ip | 1 | 0 |
| | 5 | 68.1 | ip | 2 | 0 |
| | 5 | 100.0 | ip | 5 | 0 |
| | 5 | 10.0 | ia | 4 | 0 |
| Salicylic acid | 5 | 68.1 | ip | 1 | 0 |
| | 5 | 100.0 | ip | 2 | 0 |
| | 5 | 147.0 | ip | 2 | 0 |
| | 5 | 215.0 | ip | 5 | 0 |
| *N*-Acetylanthranilic acid[b] | 5 | 100.0 | ip | 2 | 0 |
| | 5 | 215.0 | ip | 1 | 0 |
| | 5 | 464.0 | ip | 2 | 0 |
| | 5 | 1000.0 | ip | 0 | 0 |
| | 5 | 100.0 | ia | 0 | 0 |
| Anthranilic acid[b] | 5 | 100.0 | ip | 0 | 0 |
| | 5 | 215.0 | ip | 2 | 0 |
| | 5 | 464.0 | ip | 0 | 0 |
| | 5 | 1000.0 | ip | 0 | 0 |
| | 5 | 100.0 | ia | 0 | 0 |
| Acetylthiosalicylic acid | 5 | 147.0 | ip | 0 | 0 |
| | 5 | 215.0 | ip | 0 | 3 |
| | 5 | 316.0 | ip | 0 | 4 |
| | 5 | 464.0 | ip | 0 | 5 |
| Thiosalicylic acid | 5 | 100.0 | ip | 0 | 0 |
| | 5 | 147.0 | ip | 0 | 3 |
| | 5 | 215.0 | ip | 0 | 4 |
| | 5 | 316.0 | ip | 0 | 5 |
| 2-Acetonylbenzoic acid | 5 | 100.0 | ip | 0 | 0 |
| | 5 | 464.0 | ip | 0 | 0 |
| | 5 | 1000.0 | ip | 0 | 0 |
| | 5 | 100.0 | ia | 0 | 0 |
| 2-Methylbenzoic | 5 | 100.0 | ip | 0 | 0 |
| | 5 | 464.0 | ip | 0 | 0 |
| | 5 | 1000.0 | ip | 0 | 0 |
| | 5 | 100.0 | ia | 0 | 0 |

[a] ip = intraperitoneal, and ia = intraarterial. [b] See discussion.

In contrast to other methods, the bradykinin injection causes no injury and shows that the receptors for pain are chemosensitive (18). The sensitivity and reliability of this method are indicated by the fact that one could show the difference in potency of acetylsalicylic acid and salicylic acid (Table II). The procedure of injecting normal saline prior to injecting bradykinin allows one to compare the effects of nonstimulating and stimulating agents and, therefore, increases the sensitivity of the test.

The concept of isosterism has undergone several changes since its introduction in 1919. The term "isosteres" was first applied by Langmuir (19) to designate two molecules or ions having identical number and arrangement of electrons. Hinsberg (20) recognized even earlier that certain groups such as sulfur, viylene, and trivalent carbon and nitrogen could be interchanged in the aromatic ring without appreciably altering physical and chemical properties. Hinsberg's designation of such groups was "ring equivalents." Hückel (21) included other types of inorganic and organic groups under the equivalent designation, equating methyl, methylene, and methyne to —F, —N—, and —O—, respectively.

Grimm (22, 23) applied the term "pseudoatom" to groups such as $—NH_2$ and —OH since, in his opinion, addition of a proton ($H^+$) to the oxide ion results in the addition of a proton to the outer shell where it reaches equilibrium at or in the outer shell, and the result is thus a pseudo-$F^-$ or the hydroxide ion. However, unlike the ring equivalents of Hinsberg, chemical properties of compounds obtained using the "hydride displacement law" might appear wholly different but may be similar in some physical properties.

Erlenmeyer (24) included both Grimm's concept of pseudoatoms and all elements of a particular group of the periodic table in his concept of isosteres, redefining the isosteres as "atoms, ions, or molecules in which the peripheral layers of electrons can be considered identical." Erlenmeyer was also one of the first to apply the isosteric principle to biological problems (25, 26).

**Table II**—$LD_{50}$ and $ED_{50}$ Values for Acetylsalicylic and Salicylic Acid Isosteres *versus* Intraarterial Injections of Bradykinin in Rats

| Compound | Intraperitoneal $LD_{50}$, mg/kg | Intraperitoneal $ED_{50}$, mg/kg | Intraarterial, $ED_{50}$, mg/kg | 95% Confidence Limits |
|---|---|---|---|---|
| Acetylsalicylic acid | — | 64.9 | — | 47.9–88.00 |
| Salicylic acid | — | 138 | — | 84.1–226 |
| *N*-Acetylanthranilic acid | — | 1000 | 100 | — |
| Anthranilic acid | — | 1000 | 100 | — |
| Acetylthiosalicylic acid | 224 | — | — | 176–285 |
| Thiosalicylic acid | 153 | — | — | 120–194 |
| 2-Acetonylbenzoic acid | — | 1000 | 100 | — |
| 2-Methylbenzoic acid | — | 1000 | 100 | — |

Since the development of more sophisticated methods of treating atoms and molecules (*e.g.*, quantum mechanics), it is recognized that the concept of isosterism disregards some important factors such as size, shape, polarity, and hybridization, and the concept is of little theoretical importance to the pure chemist because of the unpredictability of its application. However, it has great value for the medicinal chemist, since biological properties of isosteres are more similar than their physical or chemical properties.

Since isosteres often have similar biological properties, Friedman (27) introduced the term bioisosterism to apply to compounds "which fit the broadest definition of isosteres and have the same type of biological activity." He included under this term isosteres with antagonistic activities, since these compounds may be acting by a similar mechanism. The broadest definition of the term isostere would, of course, include the ring equivalents of Hinsberg, the isosteres of Langmuir, the equivalents of Hückel, and the pseudoatoms of Grimm, all of which have been found to have utility in the search for more desirable, biologically active compounds. Thus, Erlenmeyer found that the isosteres *p*-aminodiphenylamine, *p*-aminodiphenylmethane, and *p*-aminodiphenyl ether all show similar antigen activity. Halogen isosteres of the antihistamine, tripelennamine, show greater antihistaminic activity when F and Cl are substituted for one aromatic hydrogen in the parent compound (28). Several other examples of the utility of the concept of isosterism in medicinal chemistry can be found in the literature (27, 29).

It is unusual that none of the isosteres was effective in producing analgesia, especially since most of the groups are closely related in size. Apparently, the receptors are very sensitive to the electronic and steric configurations of acetylsalicylic and salicylic acids. However, it has been demonstrated again that the analgesic activity of acetylsalicylic acid is more than twofold greater than that of salicylic acid.

## REFERENCES


(1) L. C. Hendershot and J. Forsaith, *J. Pharmacol. Exp. Ther.*, **125**, 237(1959).

(2) B. A. Whittle, *Brit. J. Pharmacol.*, **22**, 246(1964).

(3) F. Guzman, C. Braun, R. K. S. Lim, G. D. Potter, and D. W. Rodgers, *Arch. Int. Pharmacodyn. Ther.*, **149**, 571(1964).

(4) J. J. R. Duthie, in "Salicylates: An International Symposium," A. St. J. Dixon, B. K. Martin, M. J. H. Smith, and P. H. N. Woods, Eds., Little, Brown, Boston, Mass., 1963, p. 17.

(5) L. Lasangna, *Amer. J. Med. Sci.*, **242**, 620(1961).

(6) R. Stockman, *Brit. Med. J.*, **i**, 597(1913).

(7) D. Lester, G. Lolli, and L. A. Greenberg, *J. Pharmacol. Exp. Ther.*, **87**, 329(1946).

(8) R. K. S. Lim, in "The Salicylates: A Critical Biographic Review," M. J. H. Smith and P. K. Smith, Eds., Interscience, New York, N.Y., 1966, p. 184.

(9) H. O. J. Collier, in "Evaluation of Drug Activities: Pharmacokinetics," vol. I, D. R. Laurence and A. L. Bacharach, Eds.,

Academic, New York, N.Y., 1964, pp. 183–203.
(10) G. Deffenu, L. Pegrassi, and B. Lumachi, *J. Pharm. Pharmacol.*, **18**, 135(1966).
(11) "Organic Syntheses," coll. vol. II, A. H. Blatt, Ed., Wiley, New York, N.Y., 1961, pp. 580–583.
(12) A. I. Vogel, in "A Text-Book of Practical Organic Chemistry," 3rd ed., Longmans, Green & Co. Ltd., London, England, 1959, p. 175.
(13) *Ibid.*, p. 176.
(14) O. Hinsberg, *Chem. Ber.*, **43**, 654(1910).
(15) J. Schnekenburger, *Arch. Pharm.*, **297**, 734(1964).
(16) *Ibid.*, **298**, 4(1965).
(17) W. R. Thompson, *Bacteriol. Rev.*, **11**, 115(1947).
(18) R. K. S. Lim, in "The Salicylates: A Critical Bibliographic Review," M. J. H. Smith and P. K. Smith, Eds., Interscience, New York, N.Y., 1966, p. 155.
(19) I. Langmuir, *J. Amer. Chem. Soc.*, **41**, 1543(1919).
(20) O. Hinsberg, *J. Prakt. Chem.*, **93**, 302(1916).
(21) W. Hückel, *Z. Elektrochem.*, **34**, 430(1928).
(22) H. G. Grimm, *ibid.*, **31**, 474(1925).
(23) *Ibid.*, **34**, 430(1928).
(24) H. Erlenmeyer, *Bull. Soc. Chim. Biol.*, **30**, 792(1948).
(25) H. Erlenmeyer and E. Berger, *Biochem. Z.*, **252**, 22(1932).
(26) H. Erlenmeyer, E. Berger, and M. Leo, *Helv. Chim. Acta*, **16**, 733(1933).
(27) H. L. Friedman, in "Symposium on Chemical-Biological Correlation," National Academy of Sciences–National Research Council, Publ. No. 206, Washington, D.C., 1951, p. 295.
(28) J. R. Vaughan, G. W. Anderson, R. C. Clapp, J. H. Clark, J. P. English, K. L. Howard, H. W. Marson, L. H. Sutherland, and J. J. Denton, *J. Org. Chem.*, **14**, 228(1949).
(29) V. B. Schatz, in "Medicinal Chemistry," 2nd ed., A. Berger, Ed., Interscience, New York, N.Y., 1960, p. 72.

**ACKNOWLEDGMENTS AND ADDRESSES**

Received July 29, 1974, from the *Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, CA 94143*

Accepted for publication October 11, 1974.

* Present address: Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46206

ˣ To whom inquiries should be directed.

# Fluorocarbon Aerosol Propellants IV: Pharmacokinetics of Trichloromonofluoromethane following Single and Multiple Dosing in Dogs

**SARFARAZ NIAZI and WIN L. CHIOU** ˣ

**Abstract** □ An intravenous dosage form of trichloromonofluoromethane, a common aerosol propellant, was formulated in polyethylene glycol 400 for single and multiple dosing to unanesthetized dogs. A three-compartment open model was proposed for disposition of this compound in dogs with average half-lives of 3.2, 16, and 93 min for three disposition phases. This finding is contrary to several reports where blood levels were monitored for shorter periods. A computer analysis of tissue compartment distribution following a single dose showed that about 2 hr was required to achieve pseudodistribution equilibration, following which more than 90% of the dose remaining in the body was retained in tissue compartments. Pulmonary clearance and volumes of distribution were calculated considering first-pass effect through the lung. The volume of distribution was approximately six times the body weight in terms of blood concentrations, and about 30% of the propellant was cleared from blood passing through the lung in each cycle. Disposition of propellant followed dose-independent kinetics after multiple dosing, and accumulation in tissues continued for a much longer period, resulting in high tissue compartment levels.

**Keyphrases** □ Trichloromonofluoromethane—pharmacokinetics following single and multiple dosing, dogs □ Fluorocarbon aerosol propellants—pharmacokinetics of trichloromonofluoromethane following single and multiple dosing, dogs □ Aerosols—pharmacokinetics of trichloromonofluoromethane following single and multiple dosing, dogs □ Propellants—pharmacokinetics of trichloromonofluoromethane following single and multiple dosing, dogs □ Pharmacokinetics—trichloromonofluoromethane, single and multiple doses, dogs

The volatile fluorocarbons have been widely used as aerosol propellants in commercial aerosol packages in this country. Although these compounds have been generally considered nontoxic and inert, several studies (1–3) have claimed that a variable degree of cardiac damage can be caused following their inhalation. An often used argument in favor of low toxicity of fluorocarbons is that these compounds are not absorbed to any significant extent when inhaled from a commercial aerosol product and that the small fraction absorbed is eliminated very fast from the body, decreasing the possibility of any toxic reaction (4–6). Unfortunately, the conclusions drawn from most previous studies monitoring blood levels of fluorocarbons following their inhalation from commercial aerosol products were not based on sound pharmacokinetic principles and do not reflect the true disposition pattern of these compounds as was recently discussed (7).

The objective of the study reported here was to demonstrate that, contrary to the established belief, trichloromonofluoromethane, one of the most commonly used fluorocarbon propellants, has a longer biological half-life than previously thought and under-