Development of a Mammalian Screen to Evaluate Absorption and Persistence of Fluorinated Chemicals

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Abstract

Questions have arisen regarding the potential of fluoroorganic chemicals to be absorbed and to bioaccumulate in the human body. Rats were used in an experiment to measure the blood uptake and clearance for a series of fluoroorganic chemicals. Eighteen chemicals were screened at doses expected to be minimally toxic. Each chemical was administered by oral gavage to groups of 5 rats each for 10 days. Blood was collected 2 hours after the first dose, and on days 5, 10 (treatment), 13, 24, 52, and 94 (post-treatment). The total fluorine content (ppm F) in blood was determined by using a Wickbold torch combustion/fluoride ion electrode analysis method. Noncompartmental analysis was conducted on blood fluorine data. The dose-normalized area under the blood-concentration curve (AUC/D) was calculated for each chemical and used as a comparative metric. The blood AUC/D for the chemicals tested ranged from 566,500 for perfluorooctane sulfonate (PFOS) and 70,800 for perfluorooctanoic acid (PFOA) to a range of 0 to 28,900 for a series of Telomer-based chemicals. This screen could also be used to assess levels of test material derived fluorine in target organs or estimate the biological half-life of test materials. As utilized here, the screen proved valuable in assessing the relative biopersistence of a wide range of fluorinated chemicals.

Introduction

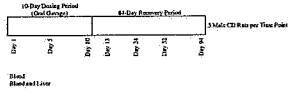
The objective of these studies was to examine the potential of selected fluorinated organic chemicals to be absorbed and persist in a mammalian system. This rat assay was developed as a screening test that could be used to compare bioaccumulation/biopersistence potential of the chemicals to one another. Blood samples taken from rats at selected time points during a 10-day dosing phase and an 84-day recovery phase were analyzed for total fluorine. The data were normalized so that comparisons could be made, regardless of chemical characteristics or the dose administered. Non-compartmental kinetic analysis of the blood data was used to provide the basis for comparisons. Liver samples were also analyzed for total fluorine to supplement the information obtained from blood analyses.

Methods

- **Animals**
  - Sprague Dawley male rats
- **Test Materials and Dosages**
  - Selected fluorinated organic chemicals were given to rats by gavage at the indicated dosages. The doses were selected to achieve minimal toxicity (some effect on body weight gain) or an upper limit of 1000 mg/kg/day.

Identifier	Chemical	Dosage (mg/kg/day)
A	Perfluorooctane sulfonate	10
B	Ammonium perfluorooctanoate	20
C	Fluoroalkylethyl Sulfonate	35
D	Fluoroalkylethyl Betaine	75
E	Fluoroalkylethanol Mixture	100
F	Fluoroalkylethyl Carboxylate	100
G	Mixture of Fluoroalkylethyl Anionic Surfactants	200
H	2-Perfluorooctylethanol	300
I	Fluoroalkylethyl Ethoxylate	300
J	Fluoroalkyethyl Methacrylate Polymer	1000
K	Fluoroalkylethyl Acrylate Polymer	1000
L	Fluoroalkylethyl Phosphate #1	1000
M	Fluoroalkylethyl Phosphate #2	1000
N	Fluoroalkylethyl Urethane Polymer	1000
O	Polytetrafluoroethylene	1000
P	Polytetrafluoroethylene mixture	1000
Q	Perfluoropolyether #1	1000
R	Perfluoropolyether #2	1000

- **Vehicles and Negative Controls**
  - The test chemicals were administered as received or were suspended in water, corn oil, or a mixture of acetone and corn oil (20:80). Negative control rats were dosed with water or respective carriers.
- **Study Design**



- **Total Fluorine Analyses**
  - Wickbold torch combustion followed by analysis with selective ion electrode
- **Data analyses**
  - All data were normalized to 0.1 mmole/kg based on dosage, percentage active ingredient, and percent fluorine, to compare kinetics from one material to another.

The following parameters were calculated for the blood:

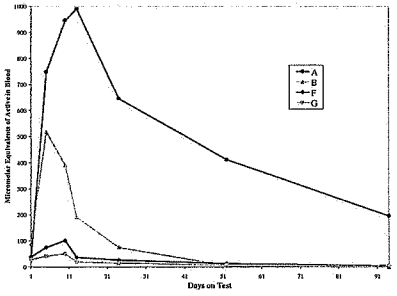
AUCINF/D (area under the curve) -- Represents the area under the blood concentration curve from the time of initial dosing extrapolated to infinity.

Cmax -- Maximum observed concentration in the blood

Results

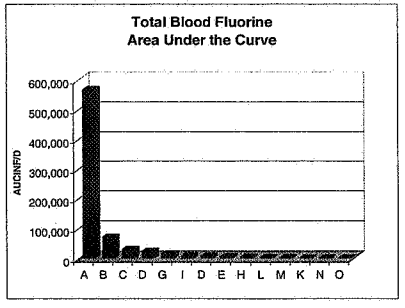
- **Magnitude of Response Comparisons**
  - A plot of total organic blood fluorine concentration normalized to micromolar equivalents vs. time provides a comparison of the magnitude of Cmax and area under the curve for selected chemicals. In this example, four selected chemicals are shown:

Chemical	AUCINF/D	Cmax
A	566,479.1	989.1
B	70,789.6	518.1
F	23,298.6	101.1
G	14,886.1	49.3

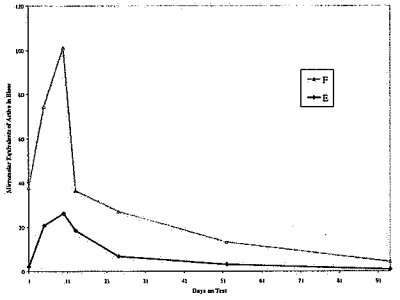


- **Comparison of AUCINF/D for Blood Organic Fluorine Across Tested Chemicals**

The area under the curve calculation provides an estimate of total fluorine load in the rat during the course of the dosing and recovery periods. Fourteen of the tested 18 chemicals are compared for relative accumulation in a mammalian system. The other four chemicals tested had no measurable area under the curve.

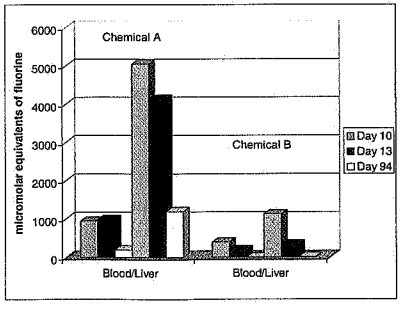


- **Determination of Uptake and Clearance**
  - A plot of blood fluorine levels vs. time illustrates the rate of absorption and clearance from the blood. In this example, absorption and clearance patterns of two selected chemicals are compared. Fluorine blood levels for Chemical F do not reach steady state within the 10-day dosing period in this test. No estimate of steady state level is available. Alternatively, total fluorine levels for Chemical E indicate that the chemical may be approaching steady state near the end of the dosing phase. Differences in clearance patterns can also be seen. Initial clearance of Chemical F from the blood is rapid after dosing ceases and is followed by slower clearance between days 13 and the end of the recovery phase. Clearance of Chemical E follows a different pattern and appears to be slower.

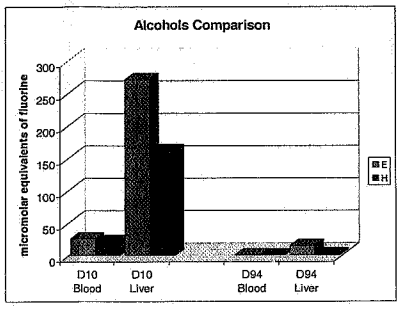


- **Comparison of Organic Fluorine Levels in Blood and Liver**

The Wickbold torch can be used for combustion and subsequent analysis of total organic fluorine in tissue samples. In the first example, fluorine levels in blood and liver of animals treated with Chemicals A and B are compared at the end of dosing (Day 10), three days after the dosing phase (Day 13) and at the end of the recovery phase (Day 94). Both blood and liver samples have higher levels of organic fluorine in rats treated with Chemical A than in those treated with B.



- In the second example, two alcohols are compared at the end of the dosing period (Day 10) and at the end of the recovery period (Day 94). Blood levels were similar, but levels of fluorine in the liver were higher for one alcohol than for the other.



Discussion

- Normalization of data by taking into consideration the dosage administered and the organic fluorine content of the test chemical makes it possible to readily compare different test materials. Kinetic analysis and calculation of AUCINF/D for this set of chemicals showed a wide range in the potential for fluorine to accumulate in the blood of treated rats. Area under the curve ranged from a high of approximately 566,500 for Chemical A (PFOS) to a low of zero. Fluorotelomer process chemicals had AUCINF/D values of 28,900 or less in this assay. It is recognized, however, that not all of these chemicals had achieved steady state in the blood by the termination of dosing on Day 10. Thus, the calculated numbers have limitations and should be used accordingly.
- To determine if organic fluorine can be stored in other body compartments, tissues were also combusted and analyzed by this method. The fluorine levels in the liver indicated that blood levels do not account for the total fluorine storage in the body. A variety of liver levels were evident, indicating a need for further assessment of distribution for some of these materials. The Wickbold torch method of analysis is slow and tedious, but the measurement of total organic fluorine can show accumulation in tissue without having specific analytical methods available for each parent or metabolite. It is also useful when evaluating the results of exposures to mixtures, which make up a large portion of this chemical selection.

Conclusion

This mammalian screening study was used successfully to rank a series of fluorinated chemicals for bioaccumulation/biopersistence potential. The calculated blood area under the curve for perfluorooctane sulfonate (PFOS) was 8x that of ammonium perfluorooctanoate (APFO) and 19-1800x that of fluorotelomer-based materials. This screen has the potential to be a valuable tool for use in selection of chemicals for further research and in development of experimental designs.

Acknowledgements

The authors would like to thank the following:

- Study technicians
  - Nita Baker, Rich Mathena, Steve Carpenter, Charlene Smith
- Fluorine analysis
  - Chris Bowers and Ward Gibson
- Kinetic analyses
  - Paul Hinderliter
- Poster Preparation
  - Maryanne Wilford