United States
Environmental Protection
Agency

Health Effects Research
Laboratory
Research Triangle Park NC 27711

Research and Development

EPA-600/S1-83-002 Apr. 1983

EPA

Project Summary

Development of a Model of Toluene Blood Level Following Subcutaneous Injection of Toluene in the Rat

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A model of toluene level in blood following subcutaneous (SC) injection of toluene mixed with polyoxyethylated vegetable oil vehicle was developed. The purpose was to provide a means of predicting dose received, for subsequent toxicologic studies for any time and dose combination. The pharmacokinetics were of secondary interest. With the use of data from 111 rats, a 4-parameter equation was devised to predict the course of toluene blood levels from 20-480 minutes, for doses of 50-1000 mg/kg. Blood concentrations rose at a rate that was independent of dose level. Maximum blood levels were uniquely determined by dose level. Blood levels fell at differing rates, depending upon dose level. When compared to inhalation, injection exposure has the advantages of low expense, low equipment requirements, and simplicity. Its disadvantage for some experiments is poor temporal simulation of the normal route of exposure, inhalation.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Toluene is a commonly occurring substance, widely used as a solvent in paints, glue, and rotogravure printing, to name but a few applications. Threshold concentrations for effects on neural and behavioral functions range from 100 ppm in ambient air to about 1000 ppm, depending upon the dependent variables.

Humans are normally exposed to toluene via inhalation. Injection has been used by many investigators as a convenient and inexpensive alternative to the more complex technology of inhalation exposure. Despite the common use of this method, only one study was found in which tissue levels of toluene after injection were reported (Sato and Nakajima, 1979). Using intraperitoneal (IP) injections of 30, 115, and 460 mg/kg, the researchers measured blood levels at 2, 4, 8, 12, and 24 hours in rats. Although most of the rising limb of the curve occurred before the first blood sample was taken, it appeared that toluene reached its peak blood value later for larger doses. Since statistical tests were not performed, it was not clear whether the elimination rate was a function of dose level. Sampling rate for blood level was also quite low. Due to infrequent and late sampling and lack of statistical testing of the curve fits, it is unclear whether the curves which these investigators used adequately described the time course of blood levels.

Toluene has been injected both IP and SC by various investigators, but blood levels over time were not reported. For many experiments, it is desirable for blood toluene to be maintained at a stable level

for a long period of time rather than to reach a peak and to be eliminated rapidly. Drugs delivered by SC injection are more slowly and evenly absorbed over time than are those given by IP injection. No data were found, however, on toluene blood levels after SC injection.

This study was performed to discover and describe the time course of toluene in blood after injection, because the design of subsequent toxicologic studies would be greatly simplified if such information were available. The pharmacokinetics were of secondary interest. SC injection was used because a long, slow absorption is often desirable. If the time course of toluene blood level after injection were known, preliminary study could be conducted using injections rather than the more expensive and complex inhalation procedure.

Materials and Methods

The work in this report was an alternating series of exploratory and confirmatory studies. The process of devising the injection technique and the model for predicting results is described in detail in the "Results" section of the full publication.

All 254 subjects were Long-Evans hooded rats of similar age. All rats were kept in a colony on a night-dark schedule for a minimum of 30 days before use.

Injections were administered SC after the rat was grasped with the non-dextrous hand and the skin of its back was pushed upward to form a "hump." A 22-gauge, one-inch needle on a plastic syringe was inserted into the skin hump, in an anterior direction parallel to the back of the rat. After the injection was complete, the needle was withdrawn and the injection site was rubbed in a posterior to anterior direction 10 times in order to avoid leakage from the site.

Toluene was mixed in various ratios with polyoxyethylated vegetable oil (Emulphor, EL610) to achieve an injection volume of 1.6 ml/kg of body weight. Dosages were 50, 100, 120, 150, 500, and 1000 mg/kg.

Rats were injected in the morning and then housed in individual cages in a lighted room without food or water until they were sacrificed. Rats were rendered unconscious by cervical dislocation, and 3.5 ml of blood was drawn from the anterior vena cava as described in Benignus *et al.* (1981). Rats were sacrificed at 20-minute intervals after injection for up to 8 hours. Gas chromatography was used to analyze the blood samples (Benignus *et al.*, 1981).

Previous data have shown that variance of blood levels is much greater for higher doses than for lower doses. Exploratory analyses in this study confirmed this. Consequently, all data were analyzed in log concentrations. The models of most interest are inherently nonlinear. Methods used for fitting inherently nonlinear equations were programmed in Statistical Analysis System. When parameter estimates for different nonlinear equations were to be compared, the technique of seemingly unrelated nonlinear regression was used.

Conclusions

The time course of toluene in blood following SC injection was observed (see Figure 1). Rise rates, maximum blood level, and decline of toluene in blood were all functions of dose level. The following 4-parameter equation was devised to predict blood level as a function of time after injection and dose level:

$$\log C_i = (\log 1.58)(\log D_i) + \log[1-\exp(.0106\, t_i)] - t_i(.00838 - .0012 \log D_i). \quad (1)$$

Here C_i = blood toluene (ppm) for rat i, D = dose of toluene in mg/kg, and t = time after injection. The purpose was to enable predictions of dose received in subsequent toxicologic research without investigations of the pharmacokinetics.

The SC injection technique appears to have utility as a method of toluene exposure. Its advantages are its low expense and equipment requirements, as well as its simplicity. Its disadvantage is its poor temporal simulation of inhalation exposure for certain kinds of studies. Perhaps it would be most useful in conducting preliminary work on the effects of toluene. Effects would have to be verified with inhalation exposures in order to generalize the results to the usual route of exposure, inhalation.

References

Sato, A and T. Nakajima. Dose-dependent metabolic interaction between benzene and toluene *in vivo* and *in vitro*. *Toxicol. Appl. Pharmacol.* **48**:249-256, 1979.

Benignus, V., K. Muller, C. Barton, and J. BittiKofer. Toluene levels in blood and brain of rat during and after respiratory exposure. *Toxicol. Appl. Pharmacol.* **61**:326-334, 1981.

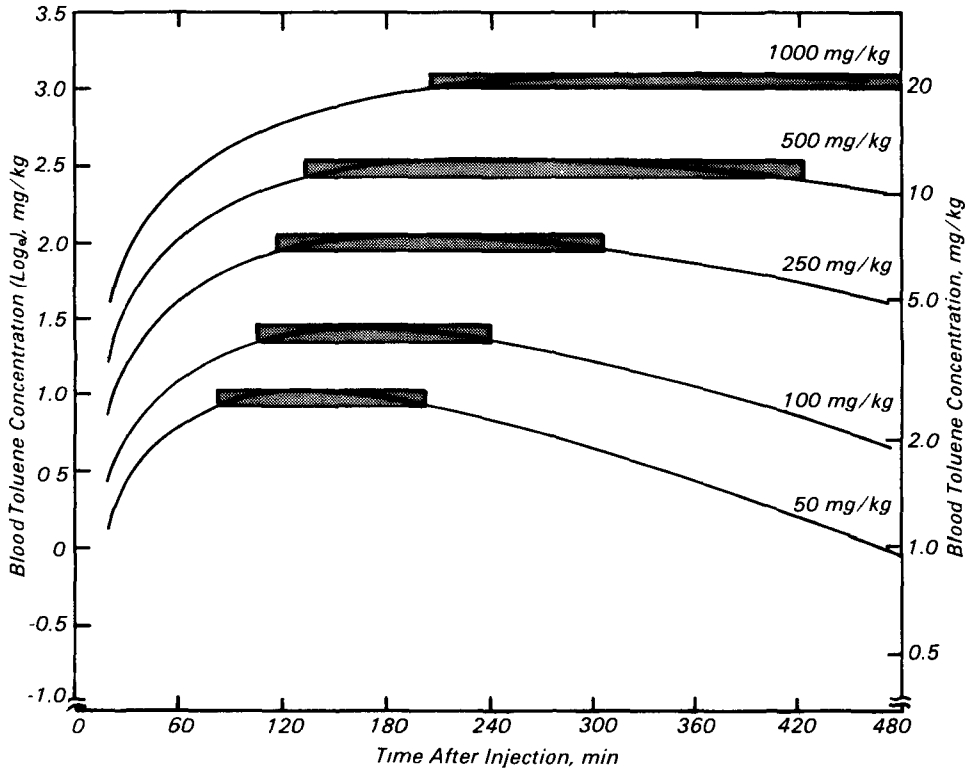

Figure 1. *Lines of best fit for the 4-parameter model. The crosshatched bars show the times during which the toluene concentration in blood is estimated to be at least 90% of maximum (raw data) for each dose.*

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The complete report, entitled "Development of a Model of Toluene Blood Level Following Subcutaneous Injection of Toluene in the Rat," (Order No. PB 83-172 494; Cost: $10.00, subject to change) will be available only from:

National Technical Information Service
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☆U.S. Government Printing Office: 1983-659-017/7056

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