EPINEPHRINE CHALLENGE FOR CARDIAC SENSITIZATION TESTING VS. ENDOGENOUS EPINEPHRINE: A PERSPECTIVE

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BACKGROUND AND DISCUSSION

The basis of the cardiac sensitization test is to determine the level of exposure to a chemical that results in anomalous heartbeats, which, if allowed to continue, would be life threatening. The test is conducted using dogs trained to tolerate the test procedure. Each dog is tested to determine the dose of epinephrine (1 to 12 µg/kg body weight) that would just evoke *a* cardiac response (unifocal ectopic beats). This dose is then used for testing the cardiac sensitization potential of a halon replacement candidate. The cardiac sensitization test involves 10 min exposures to sequentially increasing doses of the test chemical with an epinephrine challenge given at 5 min of exposure. A positive response is one that expresses multifocal ventricular ectopic activity. The lowest dose **at** which cardiac response is observed is designated the LOAEL (lowest observed adverse effect level). The next lowest dose tested. at which no positive response is observed, is designated the NOAEL (no observed adverse effect level). These endpoints are determined in animals already primed for a cardiac response by virtue of the high level of epinephrine challenge. The concentration of the exposure would have to be much higher to evoke the response without an external epinephrine challenge. EPA applies the LOAEL and NOAEL endpoints gained from these dog tests directly to humans because of recognition of the high sensitivity of the test procedure. Thus the LOAEL and NOAEL levels established by this test procedure are very conservative estimates of the levels that would evoke a cardiac response without an external source of epinephrine. In spite of the declared conservative nature of the test. the question often raised is whether a person caught in an exposure situation to one of these chemicals at a level at or above the LOAEL will respond with a life-threatening cardiac response. The key to answering this question lies in demonstrating two things. First. a comparison must be made between (a) the level *of* circulating epinephrine resulting from a challenge during a cardiac sensitization test and (b) the maximum level of circulating endogenous epinephrine, which may result from extreme exertion during exercise or from an anxiety/stress situation. Second, a demonstration must be provided of the levels at which a cardiac response may occur if an external epinephrine challenge is not given.

Epinephrine, a naturally occurring chemical in the body, can evoke cardiac responses if external sources of epinephrine are injected into the bloodstream. The levels of epinephrine necessary to evoke a cardiac response are much higher than those that can be generated under normal physiological conditions of stress or exercise. The range measured in a diverse group of mammalian species (rat. mouse, rabbit, dog, cat, monkey, pig. cow) is from less than 0.1 to nearly 0.6 µg/L under conditions of physiological states going from rest to some degree of activity [1]. Levels measured in man from conditions of rest to extreme activity varied from **less** than 0.03 to slightly over 1.0 µg/L [2]. In an attempt to see the full range of attainable levels of epinephrine, a study was performed with Sprague-Dawley rats **[3]** in which indwelling cannulae were placed prior to experimentation. Blood was drawn under conditions of sleep, undisturbed wakefulness, handl-

ing, immobilization, and post-decapitation. Under the most extreme state of fright (immobilization) epinephrine reached a level of 2.4 µg/L. The abnormal condition of decapitation produced a maximum dump of epinephrine resulting in a concentration of 15.0 µg/L.

Stressed exercising dogs reached a level of circulating epinephrine of about 0.4 µg/L [4,5,6,7]. During cardiac sensitization testing dogs are challenged with doses of epinephrine ranging from 1.0 to 12.0 µg/kg body weight [8]. Since dogs have a total blood volume of about 8.2% body weight [9] the stated doses of epinephrine would result in concentrations of about 12 to 146 µg/L or 30 to 365 times the levels circulating under normal stressed physiological conditions. These are conservative estimates of the concentration that reaches the heart with an epinephrine challenge since the above calculation assumes that the epinephrine immediately dilutes throughout the whole blood volume. The actual dilution, between the site of injection into the cephalic vein and reaching the heart muscle through the coronary artery, is into about one-fifth (20%) of the blood volume. Concentrations reaching the heart through the coronary arteries would be five times higher than calculations above indicate (60 to 730 µg/L or 150 to 1825 times the circulating levels).

The relationship between epinephrine concentration and concentration of chemical that produces a cardiac response is illustrated in Figure 1. This figure is meant to show relationships and does not present actual numeric values. Epinephrine concentration varies from baseline resting physiological levels to levels reached only by giving exogenous epinephrine. **As** noted above, these higher levels conservatively vary from 30 to 365 times endogenous levels even under conditions of stress. Thus the upper concentration of the physiological range of epinephrine is well below that attained during an exogenous epinephrine challenge as illustrated in the figure. The **LOAEL** is currently determined with an injected dose of epinephrine that is just below that which would

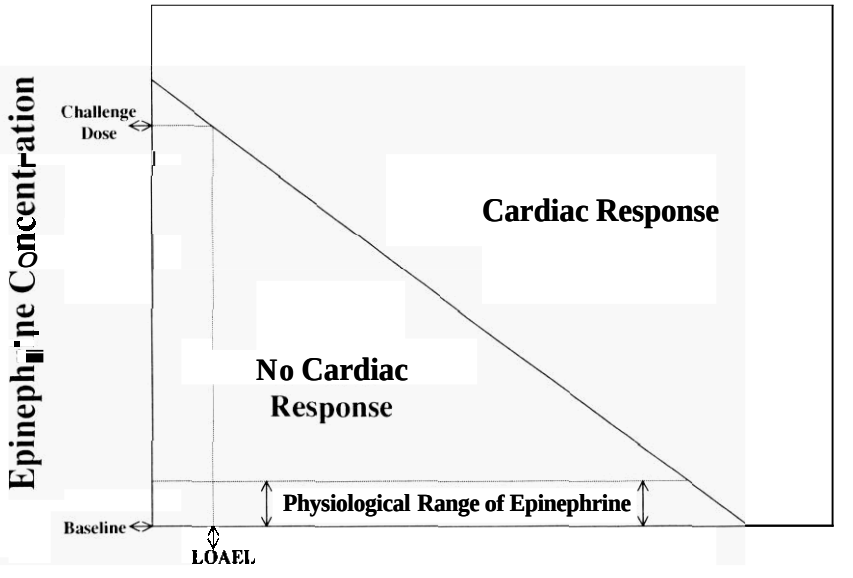

Figure 1. Relationship of epinephrine concentration to target concentration of chemical for cardiac response.

produce a cardiac response even without a chemical challenge. Thus, the LOAEL for a given chemical is the result of the epinephrine level chosen for the test. If the cardiac sensitization test were done with a lower level of epinephrine, the resulting LOAEL would be higher. Ultimately. how much lower an epinephrine challenge could he used would depend upon the shape of the line separating the region of "no cardiac response" from that of "cardiac response." The goal would he to he able to select a challenge concentration of epinephrine that would still be "safely" ahove the physiological range and yet result in a substantial increase in the LOAEL determined.

Why is there a need to redesign the cardiac sensitization test as it is currently performed! This need occurs because of the difference in the way the test is currcntly being used for regulatory purposes as compared to its original use. Originally, the test was developed as a way of ranking chemicals relatively for their potential as cardiac sensitizers. For this purpose. the only thing that mattered wits that the test was standardized and thus could be applied across groups of chemicals. The absolute value obtained was not as much of concern as the relative value. Now, however, the results of cardiac sensitization tests are being used on an absolute basis for determining the safe use of a chemical. Cardiac sensitization NOAEL and LOAEL values are compared to actual use levels of a chemical. and regulations are made on whether the chemical can be used where there is a possibility of human exposure. This approach has become of particular concern for replacement of ozone-depleting substances such as Halon 1301 (CF_3Br) widely used for flooding occupied areas to extinguish fires. Many of the agents proposed for replacing Halon I30I have cardiac sensitization LOAELs either below or not much higher than the extinguishing concentration of the agent. This, of course, affects the potential usefulness of the agent. Even in cases where the LOAEL may be high enough above the extinguishing concentration to consider using the agent, secondary issues may preclude the use of the agent. Many of these halogenated agents release halogen acids upon contact with heat. The acid levels generated can be highly toxic. One way to avoid the release of toxic levels of these acids is to use the extinguishing agent at higher concentrations so that the fire is extinguished more rapidly. This higher use concentration may once again approach or overlap the LOAEL for cardiac sensitization.

What current evidence is there that safe exposure to higher levels of cardiac sensitizing chemicals can occur under normal physiological conditions'? Although there are relatively few chemicals for which good exposure data exist for dogs and humans, some relevant information is available for trichlorofluoromethane (CFC-I I), dichlorodifluoromethane (CFC-12), bromotrifluoromethane Halon 1301, and trifluoroiodomethane (CF_3I).

For CFC-I I, the cardiac sensitization LOAEL is 0.3% (3000 ppm) and NOAEL is 0.1% (1000 ppm) as determined in dogs. Two male dogs were exposed to 10.250 ppm of CFC-I I for 8 hrs/day, 5 days/wk. for six weeks [I0]. No visible signs of toxicity werc noted. Dogs. trained to run on an enclosed treadmill, were exposed to concentrations of 0.5, 0.75. and I.0% of CFC-1 I for up to 16 min [I I]. Although the dogs did not tolerate the exposures well. there were no reported cardiac responses. Twelve dogs were exposed to 80% CFC-I1/20% O_2 for 30 sec with noise with two of the animals showing marked cardiac effects [12].

For CFC-12, the cardiac sensitization LOAEL is 5.0% (50,000 ppm) and NOAEL is 2.5% (25.000 ppm). Dogs were exposed to 20% CFC-I2 for 7 hrs/day, 5 days/wk and showed no signs

of severe poisoning [13*]. Dogs, trained to run on an enclosed treadmill, were exposed to concentrations of 5.0, 7.5, and 10.0% of CFC-12 for up to 16 min [11]. Two exposures were performed at 10.0% with one being reported as probably higher than 10.0%. One dog of the six exposed at this latter concentration demonstrated a questionable positive reaction. This same dog had shown a similar reaction under other testing conditions. Twelve dogs were exposed to 80% CFC-12/20% O_2 for 30 sec with noise without marked cardiac effects [12]. Humans were exposed to CFC-I2 concentrations of 4 to 11% [13†]. One subject was exposed for 80, 80, 35, and 11 min to 4, 6, 7, and 11%, respectively. A second subject was exposed for 14 min at 4% and an additional 66 min at 2%. Assorted CNS effects were observed during these exposures, but no cardiac effects were observed.

Halon 1301 has a cardiac sensitization LOAEL of 7.5% (75,000 ppm) and NOAEL of 5.0% (50,000 ppm). Dogs were exposed to Halon 1301 for 60 min at 5.0, 7.5. and 10.0% [14]. No cardiac abnormalities were observed during these exposures. In another study dogs were exposed to 10, 20, 30, 40, 50, 60, 70, or 80% Halon 1301 in oxygen [IS]. Some alteration of cardiovascular function was seen in all dogs exposed to 20-30% or higher. These alterations included changes in blood pressure and heart rate and the appearance of arrhythmias. The authors did not summarize the data in a way that would allow the assignment of a LOAEL for the type of arrhythmias that would be associated with cardiac sensitization. Central nervous system effects were observed at concentrations of 20% and higher. The severity of the effect increased at higher concentrations. Once again, the authors did not carefully describe the dose-response relationship of the effects.

CF_3I has a cardiac sensitization LOAEL of 0.4% (4000 ppm) and NOAEL of 0.2% (2000 ppm). Five dogs were exposed to CF_3I at 2.5% for 10 min and one was exposed to 5.0% for 4 min. Although the exposures were not well tolerated, no cardiac effects were noted. Two humans inhaled 100% CF_3I by taking a deep breath from a balloon and exhaling after a 5-10 sec breath hold [16]. These two individuals performed this task 15 to 17 times between them over a course of months without any noticeable discomfort.

CONCLUSION

Further elucidation of the relationship between the dose of epinephrine given during a cardiac sensitization test and the LOAEL observed for a given test agent could potentially lead to broader acceptance of agents that are considered to pose too much of a risk for cardiac sensitization potential.

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† R. A. Kehoe, "Report on Human Exposure to Dichlorodifluoromethane in Air," The Kettering Laboratory, Univ. of Cincinnati, unpublished (July 1943). (cited in Azar et al., 1972 [13]).

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