8 AEROSOL TOXICOLOGY

TOXICOLOGY has been defined recently as the science dealing with the effects, antidotes, detection and other aspects of the interaction of chemicals with various forms of life; or, more succintly, a study of the chemical facts of life. The chemicals we are talking about may be quite natural and normal in their origin, or they may be man-made substances that enter the body by ingestion, inhalation or even through the permeable barrier of the skin. In a great many instances chemicals are produced *in situ,* when the body is exposed to initiating substances or to radiation.

Toxicity and toxicology should not be confused as to their meaning. The word "toxicity" is derived from the Greek word for poison and can be defined as the inherent capacity of a substance to produce injury. A related term, "hazard", indicates the probability that substantial injury will result from reasonably foreseeable uses. Hazard is thus the antithesis of safety. All these terms are relative. Nothing is completely non-toxic or non-hazardous. Pure water can be toxic to a drowning person, or irritating to the skin after long exposure. Pure air can be toxic to an individual with a severe case of the bends, or suffering from drastic hypothermia due to overexposure to cold air. Toxicity is a matter of dosage. Many trace elements, such as chromium and selenium, are vital to continued health, but are violent poisons if taken in large quantities. Some become carcinogens at higher dosage levels.

Exposure to possible toxicity is sometimes a matter of choice, as in the smoking of cigarettes compared with non-smoking. The choice may be made by governments. For example, Canada has approved the use of cyclamates but banned saccharin in food products, whereas the reverse holds true in the U.S.A. Choices may often be made between product forms. Aerosol antiperspirants, for example, are often perceived as being more irritating to the respiratory tract than per-

sonal deodorants, and such problems can be avoided completely by the use of stick and roll-on alternates. In some instances there simply is no choice; one breathes the available air and drinks the available water, since corrections are either long term or unacceptable for various reasons.

The toxicology of a substance must be examined from a large number of aspects in order to assure relative safety in use. For example, mineral oils and paraffins are used widely in topically applied products and sometimes in foods, but will produce lipid pneumonia when finely aerosolized and inhaled. Petroleum distillates also produce lipid pneumonia, while vegetable oils may bring about eosinophil pneumonia when atomized and breathed. Silicon dioxide (sea sand), when finely pulverized, is safe orally and topically, but prolonged exposure to even small amounts of the dust by inhalation causes silicosis (progressive pneumoconiosis). Aerosols are unique in that they may atomize rather safe materials that would never otherwise be inhaled. As a result, inhalation toxicology is an extremely important aspect of the toxicology of aerosols.

Most toxicity experiments are divided into two main types: acute or short-term studies and chronic or long-term tests. Recently the time differences between the two have blurred. New terms, such as sub-chronic testing, have been introduced to cover programs that take from about two-weeks to six months to complete in the laboratory. The longest chronic studies may require up to about 42 months: to 6 months for protocol development and other preparations; 24 months for exposure to rodents and/or other animals; and 12 months for pathological and histiopathological examination of organs, report writing and so forth. Since these massive undertakings involve perhaps 2,000 mice, rats, hamsters, dogs or combinations of mammals and take three or four years to complete, they are done only in the case of important industrial chemicals. Methylene chloride was tested in this manner during the years 1977-1981, because of early concerns expressed by the FDA. Dimethyl ether is now being tested similarly. Since these programs cost from $3 to $5 million each (1982 dollars) it is becoming quite common that companies with a stake in the outcome will divide up the costs.

Acute Toxicity

In toxicology, the term "acute" means that exposure to a substance is limited to one day or less. In fact the dosage may be given all at one brief period. The results of acute studies are often given as LD_{50} (a lethal dose for 50% of the test animals) or as LC_{50} (a lethal concentration of gas or vapor in air, for 50% of the exposed animals). Related terms are ED_{50} and EC_{50}, the effective dose or concentration needed to bring about dizziness, irritation or some biological response other than mortality. Large numbers of animals are normally used to establish all these 50% levels with some statistical accuracy. If a small test program is planned, perhaps as a pilot study, the term ALD (average lethal dose) may be obtained on the basis of only six animals.

These results, obtained, as they say, "at the drop of a rat", are useful to aerosol people only in a rough screening sense. An acute toxicity rating does not describe fully the safety or hazard of a substance. It fails to take into account the inhalation of essentially nonvolatile liquids and finely divided solids. It does not consider long-term effects, such as cumulative depositions. Or that old, weak or diseased persons may have much less resistance than a group of young, healthy rabbits. And finally, that the toxicology of a complete aerosol formula may be quite at variance with the weighted average toxicity of its component chemicals.

Slightly over 60% of all U.S.A. aerosol products are regulated by the Consumer Product Safety Commission (CPSC), under its administration of the Federal Hazardous Substances Act (FHSA) and other laws. The CPSC requires that household products exhibiting special toxicity hazards must have these hazards identified on the label. Precautionary statements are normally included, telling the consumer how to avoid the hazard, and what to do if a toxicity problem arises as a result of misuse. In the formulation of such products certain relatively hazardous ingredients may be needed. If over 10% petroleum distillates, or 4% methanol, or 2% of sodium or potassium hydroxides are included, the product label must identify the presence of these items and list hazards that may occur. Turpentine, certain acids and a few other ingredients are handled similarly. In addition, household products should be tested clinically, using the protocols in the regulation as minimums. The clinical studies are:

Toxicity (Oral, inhalation and dermal)	CPSC 1500.3(b)(5, 6)
Irritation (Skin and eyes-primary)	CPSC 1500.3(b)(8)
Corrosivity (To living tissue)	CPSC 1500.3(b)(7)
Strong Sensitizer (To living tissue)	CPSC 1500.3(b)(9).

The regulations do not actually specify that the studies be conducted, but any improperly labeled products are made subject to official actions, such as seizure and penalties. The rather costly skin sensitization test is not performed commonly unless there is reason to believe a problem may exist based upon background information. In 1982 the minimum cost of having the toxicity, irritation and corrosivity tests performed by a recognized clinical laboratory was about $1,500. Detailed 1981 prices are given in Table VIII.

Another commanding reason for having such tests performed is the growing incidence of consumer complaints relating to clinical, flammable or other safety aspects of specialty products. One major marketer in the U.S.A. reported a growth of from 5,000 consumer complaints in 1970 to almost 15,000 in 1980.

In 1981, the *British Medical Journal* reported the major categories of acute poisoning in the U.K. for the years 1971 and 1979, showing a 117% apparent increase. This data is given in Table I.

The apparent growth rate of consumer complaints in the U.S.A. and Canada has been even faster, although here, as in the U.K., products have been made even safer over the past decade.

The root causes of the problem are complex, but much of it can be related to the increasingly bad image of the chemical industry, as the result of poor coverage by the media. Press reports that are incorrect, scientifically unsound and often biased negatively may be good for the fourth estate, but they gradually act to erode public confidence in industry. Nowhere is this more evident than in the chemical industry, which is now ranked lowest of all in public esteem.

In 1981, only 29% of the general public and 43% of the thought leaders were favorably inclined to the chemical industry. Only 6% of the public and 4% of the thought leaders recognized that it makes the biggest contribution to the national economy, and a mere 12% of the public felt that it makes the largest contribution to the "quality of life". Over 61% of the public and a clear majority of thought leaders condemned the chemical industry as having the biggest problems in the health, safety and environmental areas. Despite public opinion, the facts are given in a recent report by the U.S. National Safety Council: that the chemical industry has the nation's second best record of safety and health, just behind the aircraft industry.

TABLE I

Major Categories of Acute Poisoning, 1971 & 1979

As reported to National Poisons Information Services, covering the United Kingdom and Republic of Ireland

	Reported Episodes			
	1971		1979	
Product Category	Number	%	Number	%
Drugs	8,799	49.6	19,486	50.7
Household	5,392	30.4	11,124	29.0
Agricultural	1,110	6.3	2,275	5.9
Industrial	578	3.3	2,192	5.7
Plant and Animal	1,277	7.2	2,179	5.6
Miscellaneous	578	3.3	1,170	3.1
Total	17,734		38,426	

The aerosol industry, which is really a microsegment of the vast chemical industry, has suffered a similar erosion of public confidence. As people become convinced that products are hazardous or unreliable, they look upon them more critically and increasingly report what they feel may be problems. Particularly during the period of about 1978 to the present time (1982), complaint statistics maintained by a number of marketers have shown a doubling or more of the frequency. The rise in complaint level does not seem to be product or package oriented. (See Table III of the Aerosol Marketing chapter.)

A 1981 U.K. survey showed that 29% of hair spray users and 33% of air freshener users made spontaneous criticisms of aerosols when asked of their general feelings about the products. For the hair spray users 7% felt aerosols were harmful to health, as an unsolicited comment. When asked if aerosols were harmful to health, 14% said yes and 12% said no. For the air freshener customers, 3% felt aerosols were health hazards, unsolicited. When directly asked if they thought aerosols were harmful to health 5% said they were and 26% replied that they were not. In both groups, adverse environmental effects ranked about equally with health concerns (the U.K. still uses CFC propellents), but the greatest disadvantage mentioned (by 23% of both groups) was that the dispensers would not spray; e.g. nozzle blockage or other problem.

In the U.S.A. a published consumer attitudinal survey was recently conducted among users of various aerosol products. Considering only the hair spray category, the results of interviews with about 2000 users, recent users and non-users are shown in Table II on the next page.

Several industry observers have looked at data such as this and have suggested that, while flammability/explosivity of aerosols may have constituted

the great challenge of the 1970s, toxicological considerations may be the most controversial issue of the 1980s. Considering only the current hair spray users, Table II suggests that 29% identified aerosols as having specific types of toxicity, against only 12% who were concerned about flammability/explosivity. Within the specified toxicity grouping, both users and non-users of aerosol hair sprays felt that inhalation toxicity was by far the most serious problem with these products.

Starting about 1977 the U.S. Environmental Protection Agency (EPA), which regulates about 12% of all domestic aerosol products, followed the lead of the CPSC regarding the labeling of insecticides, disinfectants, insect repellents, herbicides and like products according to toxicological hazard. They established Toxicity Category I, II, III and IV, according to the results of five clinical tests, as outlined in the *Code of Federal Regulations,* Title 40, Ch. 1, 162.10(h)(1 & 2):

Oral LD_{50}
Inhalation LC_{50}
Dermal LD_{50}
Eye Effects — such as irritation or corrosion
Skin Effects — such as irritation or corrosion

Toxicity Category I, where the minimum front panel precautionary language must read: "POISON. Keep out of reach of children. Read carefully cautions on back (or side) panel.", is defined on the basis of oral, inhalation or dermal toxicity (as distinct from skin or local eye effects) and will almost never be encountered in the form of aerosol pesticides. Front panel labeling for Toxicity Category II substances must begin with the word "DANGER"; while the other categories use the words "WARNING" or "CAUTION".

The EPA also requires label warnings where certain active chemicals are used, such as specific organophosphates; plus the stipulation of particular inert ingredients, such as nitrites, which may react with diethanolamine and certain other nitrogen compounds to form traces of N-nitrosamines, a few of which have now been identified as animal carcinogens. Formulas containing both nitrite and morpholine have been denied product registration on the basis that toxic N-nitrosomorpholine would probably form in the dispenser over a period of several months. Vitamin C and some other substances are said to inhibit the formation of N-nitroso compounds but it is doubtful if any aerosols have utilized this technology.

Under the provisions of the Poison Prevention Packaging Act of 1970 (PPPA), the CPSC has required the use of child-resistant closures for both specific compositions and in general those that are unusually toxic. The criteria for such packages are that they must be resistant to being opened by at least 85% of a group of not less than 200 children, aged 42 to 51 months, who are given five minutes to open the product. For those unable to do so, a visual demonstration without verbal explanation is given. After this, 80% must still be unable to open it. Conversely, at least 90% of adults must be able to open the package and resecure it within five minutes.

TABLE II

Consumer Attitudes
Problems with Aerosol Hair Sprays

	Current Hair Spray Users	Use an Aerosol Hair Spray	Use a Non-Aerosol Hair Spray
Respondant Base	912	636	464
	%	%	%
Have seen or heard of problems with aerosols	64	64	71
Problems:			
Harmful/pollute environment/ bad for ecology	15	18	17
Destroys/uses up ozone/ damages ozone layer	18	14	16
Hydrocarbons/fluorocarbons pollute air	2	1	3
Hydrocarbons/fluorocarbons endanger or destroy ozone	4	1	3
Other (unduplicated) ecological dangers	39	34	39
Dangerous or harmful to health	2	2	3
Aerosols are linked to cancer	4	4	4
Fumes are dangerous if inhaled	5	6	6
Bad for lungs/irritate/cause lung damage	14	11	14
Irritate the eyes	4	3	3
Dangerous in home/may explode/flammable	12	13	15
Other (unduplicated) health hazards	35	34	39
Won't work/won't spray/get stuck	7	9	8
Nozzle clogs	5	6	6
Wasteful/lose propellent/can won't empty	7	7	8
Other (unduplicated) problems with can	18	20	24

Source: Hair Spray Usage and Attitude Study, privately funded, April 1979.

Specific formula types mentioned in the PPPA include products with 2% or more of sodium hydroxide or potassium hydroxide (as in most aerosol oven cleaners), and those with more than 10% ethylene glycol. Where two or more sizes are offered, any one size product may be sold in a non child-resistant packaging form. The rule was designed to allow for the special needs of persons with eyesight problems, arthritis and so forth in childless households. Some marketers have used this part of the regulation too liberally, by providing their most popular package size in the standard, easy-to-open form.

Between 1970 and 1980 the number of accidental poisonings of children under the age of five dropped from 7.0 million to 2.6 million, a decrease of 63%, and fatalities dwindled to 151 in 1980. Some of these 1980 statistics related to certain pesticides, and this prompted the EPA to establish their "special packaging" rules, effective March 9, 1981. In regard to child-resistant packaging, a pesticide's toxicity places it in one of three categories: (1) those products which clearly match or exceed the toxicity criteria in the *Federal Register,* Title 40, 162.16(c)(2) and which must use child-resistant closures unless exempted, (2) products for which existing toxicity data are not precise enough to determine if child-resistant packaging is needed, and where such packaging is required until vindicating data might be developed, and (3) those products which clearly do not meet the toxicity criteria.

In practice, nearly all aerosol pesticides designed for consumer use are sufficiently innocuous that special packaging is not required. The status can be determined readily by reviewing the results of the clinical studies now required as a part of the documentation that must accompany every Application for Product Registration. In the case of those pesticides registered before such studies were mandated, the marketer may have the tests performed, or alternately follow the advice of qualified firms (such as toxicant suppliers), consultants or other knowledgeable persons regarding packaging. Should the marketer elect to use a child-resistant closure, it must conform to the established testing standards, regardless of the relative toxicity of the product.

Definitions of a "toxic substance" can be expected to vary, especially between countries; but in the U.S.A. the federal agencies and their state counterparts have agreed on two levels of toxicity, and their definitions are essentially identical, although often worded rather differently. The most recent definitions are shown here, taken from the Toxic Substances Control Act (TSCA):

a. A *"Highly Toxic Material"* means:
   i. A chemical substance or mixture that has a median lethal dose (LD_{50}) of 50 mg or less per kg of body weight when administered orally to young adult laboratory rats; or
   ii. A chemical substance or mixture that has a median lethal dose (LD_{50}) of 200 mg or less per kg of body weight, when administered by continuous contact for at least 24 hours on the bare skin of one mammalian species, preferably young adult albino rabbits; or
   iii. A chemical substance or mixture that has a median lethal concentration (LC_{50}) in air of 200 ppm by volume or less of gas or vapor, or 2 mg per liter of mist, fume, or dust when administered by continuous inhalation for at least one hour to young, adult laboratory rats.

b. A *"Toxic Material"* means:
   i. A chemical substance or mixture that has a median lethal dose (LD_{50}) of more than 50 mg/kg but less than 500 mg/kg of body weight when administered orally to young adult laboratory rats; or
   ii. A chemical substance or mixture that has a median lethal dose (LD_{50}) of more than 200 mg/kg, but not more than 1,000 mg/kg of body weight when administered by continuous contact for at least 24 hours on the bare skin of one mammalian species, preferably young, adult albino rabbits; or
   iii. A chemical substance or mixture that has a median lethal concentration (LC_{50}) in air of more than 200 ppm but not more than 2,000 ppm by volume of gas or vapor, or more than 2 mg per liter but not more than 20 mg per liter of mist, dust, fume, or dust when administered by continuous inhalation for at least one hour to young, adult laboratory rats.

For aerosols, the only significant route into the body is via inhalation. Respiration of aerosol mists appears to be connected with about 99% of all toxicological problems with aerosols—real or imagined. Some early work on inhalation toxicity of individual compounds was

done by Carpenter, C.P. et al and reported in the *J. Ind. Hyg. & Tox. 31(8),* 343 (1949), using Sherman albino rats, and noting the concentration of vapors required to kill 2, 3 or 4 out of 6 rats within a 14 day period following a 4 hour exposure. This data is given for those compounds found in aerosol formulations; see Table III.

The most toxic aerosol compounds were epichlorohydrin and formaldehyde, which had an approximate LC_{50} of 250 ppm under the conditions of test. The use of epichlorohydrin in aerosols (mainly as a water scavenger, where easily hydrolysed chemicals were also present) has now almost vanished. Typical formulation levels were 0.075%. Formaldehyde, as the 37% HCHO "Formalin" solution in water, is used at about 0.100% of the solution, or 0.037% as the gaseous compound, as a broad spectrum preservative. But now, with the recent bad publicity formaldehyde has received as a suspected low-order carcinogen, its use in aerosols is diminishing rapidly. The author is unaware of any acute toxicological problems associated with the use of either of these chemicals as aerosol ingredients.

TABLE III

Acute Inhalation Toxicity of Aerosol Propellents and Solvents

Substance	Vapor Concentration in Air (v%) ALC*	LC_0	LC_{50}	CL_{100}
P-11	—	—	2.62	~8
P-12	—	—	62	80
P-113	—	—	6	—
P-114	—	60	70	>80
P-22	—	>30	35	<40
P-134a	—	—	56.7	—
P-142b	—	—	12.8	—
P-152a	6.4	32	40	—
1,1-Difluoroethylene	12.8	—	—	—
1,1-Dichloroethylene	3.2	—	—	—
Propane	—	s.a.**	s.a.**	s.a.**
n-Butane	—	>24	34	45
isoButane	—	36	52	65
n-Pentane	—	—	11	—
isoPentane	—	—	16	—
n-Hexane	—	>6	—	—
n-Heptane	—	>3	—	—
Dimethyl ether (DME)	—	—	16.4	—
Nitrous Oxide	—	s.a.**	s.a.**	s.a.**
isoPropanol	1.6	—	—	—
Toluene	0.8	—	—	—
Cychlohexanone	0.8	—	—	—
Trichloroethylene	0.8	—	—	-
Methylene chloride	—	>4	—	—
1,1,1-Trichloroethane	—	>2	—	—
"Cellosolve" Solvent	0.4	—	—	—
"Cellosolve" Acetate	0.2	—	—	—
Methyl "Cellosolve" Acetate	0.8	—	—	—
Tetrachloroethylene	0.4	—	—	—
Carbon tetrachloride***	0.4	—	—	—
Trichloroethylene	0.8	—	—	—
Methyl ethyl ketone	0.2	—	—	—
Ethyl butyl ketone	0.2	—	—	—
Epichlorohydrin	0.025	—	—	—
Formaldehyde	0.025	—	—	—

Where two or more animals were tested the lowest results were reported. All data must be considered approximate, due to differences in chemical purity, techniques, etc.

*Average Lethal Concentration (6 rats; 2/6, 3/6 or 4/6 deaths).
**Simple asphyxiant, although narcotic and other effects may occur.
***This and a few other substances are included for comparisons, although no longer used in aerosol formulations.

Cardiac Arrhythmia

At least as far back as 1957 the medical profession began to realize that a wide variety of volatile solvents, including some aerosol propellents, were able to cause cardiac sensitization when inhaled in higher concentrations. The sensitized heart loses its normal rhythm and develops various arrhythmias, characterized by a more rapid, weaker ventricular pulsation and accompanied by a reduction in myocardial force and other hemodynamic effects. This abnormal rhythm may be reversed without damaging the heart, or it may progress to total cardiac disarray and irreversible cardiac arrest, inevitably followed by death from congestive heart failure.

Once the heart is placed in the metastable arrhythmial condition, a further stimulation, such as an unexpected loud noise, a slap or a bright light, can serve to bring about a condition of intolerable sensitization, with cardiac arrest and death. This may also be done with the injection of known heart stimulants, such as atropine and (more commonly) epinephrine, at doses of about 6 to 10μg/kg. In clinical studies designed to determine threshold concentrations needed to produce cardiac arrhythmia (EC_0) or cardiac arrest and death (LC_0), it is common practice to anesthetize dogs or other animals, to eliminate the effect of outside influences, then inject with epinephrine, and then start the exposure phase, which normally lasts only 5 to 10 minutes. A summary of cardiac arrhythmial and arrest responses is provided in Table IV.

The aerosol industry first encountered the cardiac arrest syndrome about 1960 with the introduction of a cocktail glass chiller product in California. The composition was 100% P-12. Teenagers (average age 17 years) concentrated the vapors by various means, some quite ingenious, and then breathed them deeply several times in order to achieve a psychodelic "high". The

euphoric period lasted from about 60 to 480 seconds, depending upon dose, often followed by a trough or rather depressed period. The glass chiller was quickly withdrawn, but this merely caused the youths to evaluate other products. Those high in CFC content and relatively free from messy or irritating concentrates were the primary targets. Although at least twenty different aerosol products have been reported in these episodes, the frypan lubricant (then 97% CFCs) and antiperspirant (then 90% CFCs) were used the most widely. But even such unlikely formulations as black paint aerosols (then about 55% CFCs) were implicated.

TABLE IV

Incidence of Cardiac Arrhythmia due to Propellent Inhalation
(Using dogs, anesthetized and injected with about 6μg/kg of epinephrine as a cardiac stimulant to stimulate conditions of stress response.)

Propellent Tradename	Propellent Formula	Concentration of Propellent (v%) Threshold to Produce Cardiac Arrhythmia, EC_0	Concentration of Propellent (v%) Threshold to Produce Cardiac Arrest, LC_0
P-11	CCl_3F	0.5 to 1.0*	0.5 to 1.0*
P-12	CCl_2F_2	2.5 to 5.0**	2.5 to 5.0**
P-21	$CHCl_2F$	0.5 to 1.0	—
P-22	$CHClF_2$	2.5 to 5.0	—
P-31	CH_2ClF	2.5 to 5.0	—
P-32	CH_2F_2	20 to 25	—
P-113	$CCl_2F{\cdot}CClF_2$	0.5 to 1.0	0.5 to 1.0
P-114	$CClF_2{\cdot}CClF_2$	2.5	5.0
P-115	$CClF_2{\cdot}CF_3$	15.0	—
P-123	$CHCl_2{\cdot}CF_3$	1.0 to 2.0	1.0 to 2.0
P-124	$CHClF{\cdot}CF_3$	1.0 to 2.0	1.0 to 2.0
P-132b	$CClF_2{\cdot}CH_2Cl$	0.25 to 0.50	—
P-134a	$CH_2F{\cdot}CF_3$	7.5	—
P-142b	$CH_3{\cdot}CClF_2$	2.5 to 5.0	—
P-152a	$CH_3{\cdot}CHF_2$	13.0 to 15.0	—
P-C318	$cyc.C_4F_8$	25.0	—
Carbon Tetrachloride	CCl_4	0.5	—
Methylene chloride	CH_2Cl_2	0.5	—
Propane	C_3H_8	>20.0***	—
isoButane	C_4H_{10}	10 to 20***	—
n-Butane	C_4H_{10}	10 to 20***	—
isoPentane	C_5H_{12}	5 to 10***	<15
Dimethyl Ether	$CH_3{\cdot}O{\cdot}CH_3$	20	—
Nitrous Oxide	N_2O	>80***	n.a.
Nitrogen	N_2	>80***	n.a.
Helium	He	>80****	n.a.

*Hamsters and rabbits gave threshold (EC_0) values of about 4%.
**Non-sensitized dogs gave EC_0 values of 5% and exercized dogs had EC_0 values of 10%.
***Test run on anesthetized monkeys, anesthetized and sensitized mice, dogs, etc.
****Tests run using 99.7% He and 0.3% O_2 at 122 atmos. (12.3 MPa) showed no ill-effects.
n.a. = non-applicable.

The industry reacted in a variety of ways, many of them public relations oriented, and including the education of specific thought leaders: police chiefs, high-school principals and teachers, team coaches, and public service workers, on the serious implications of "sniffing" or "huffing" aerosol products. Despite these efforts the problem continued, becoming quite notorious in the late 1960s. It was made a topic of the first formal hearing by the newly formed Consumer Product Safety Commission, held in 1973. During the early 1970s, teenagers discovered that sniffing the toluene in aerosol paints would provide a longer and more satisfying "high." By this time nearly all paints had changed from CFC to hydrocarbon propellents, so the transition to these products acted to curtail the number of deaths resulting from gross product misuse. During the 1976 to 1978 period, when aerosols were reformulated to CFC-free systems because of pending government regulations related to the CFC/ozone controversy, the problem finally went away. The "sniffing" of paints still continues and may even be increasing in the southwestern U.S.A., but few if any deaths result from this form of abuse. Similarly, medical students, hospital orderlies and other persons having access to nitrous oxide have turned to deliberately sniffing this gas for its euphoric effect. After one deep draught of the gas, plus an imbibition period of 15 to 30 seconds, users experience an exhilarating "high" lasting about 120 to 200 seconds. The state is prolonged with rebreathing. Aerosol cans containing whipped creams have been used for similar purposes. In one celebrated case, an elderly couple used up as many as 100 cans in one session, and averaged about three or four episodes per week. A number of serious side effects usually accompany this type of physical and mental abuse.

Biotransformation (metabolism)

Once they have entered the body, many chemicals are eliminated unchanged. The process is most rapid with gases or highly volatile solvents, where most of the elimination takes place via the lungs. Less volatile compounds are voided through the urine, feces and even through the skin in some cases. In a few cases bioaccumulation will occur, such as with lead compounds, which concentrate in the skeletal structure and bone marrow with serious long-term consequences. The drastic limitation of lead-containing compounds in aerosol paints relates to concerns of this type.

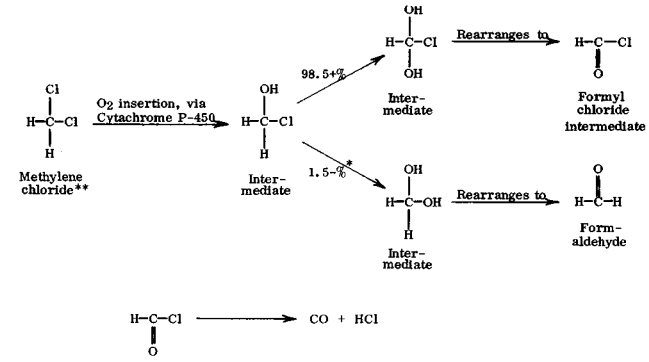


Figure 1. Biotransformation of Methylene Chloride

*Up to about 1.5% of this and other uncharacterized reactions may occur. No formic acid ($H{\cdot}CO_2H$) is formed.

**Commercial product; 97.8v% methylene chloride and 2.2v% inhibitors, not considered.

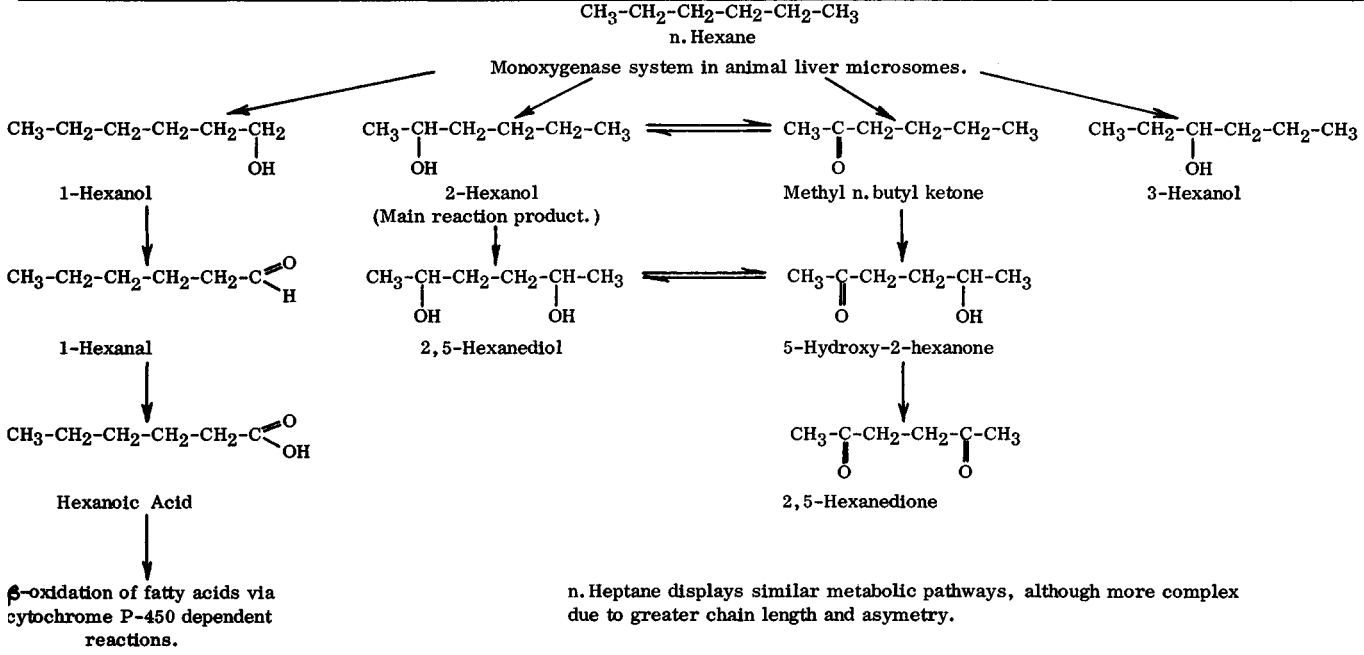
Other chemicals may be susceptible to reaction with enzymes and other biological factors, so that various percentages are metabolized into new compounds. The process is usually one of progressive oxidation, so that energy can be produced for the body. Catabolism, the formation of simpler compounds, will sometimes occur. It is a form of destructive metabolism. Conversely, anabolism (constructive metabolism) may take place, with the generation of more complex substances. Many of these processes take place in the liver in the presence of the microsomal monoxygenase system or NADPH. Cytochrome P-450 dependent reactions are involved also; for instance in the oxidation of secondary alcohols that do not undergo ordinary β-oxidation.

In the case of saturated CFCs, biotransformation is either insignificant or absent. They are excreted mainly via the lungs, more or less in relation to their volatility. The hydrogenated CFCs or FCs (such as P-152a) may be metabolized to a small extent, based upon such evidence as increased urinary fluoride. Methylene chloride, once inhaled, is 78 to 93% eliminated in the breath during the following two hours, and 98% is excreted by various routes during the first 24 hours after exposure. Approximately 94% is unchanged; the other 6% is metabolized, as shown in Figure 1.

Except for their obvious ability to oxidize by combustion, the saturated hydrocarbons are characterized by their chemical inertness; nevertheless, they can be metabolized by the body. Propane is probably biotransformed to some minute extent in animals, but no specific studies have been performed. The butanes are metabolized by rodents, but no tests have been made in man. Similarly, the pentanes, hexane and heptane are metabolized, leading to a variety of alcohols, ketones, and other compounds, as shown in Figure 2.

Figure 2. Biotransformation of n-Hexane

Note: n-Heptane displays similar metabolic pathways, although more complex due to greater chain length and asymmetry.



Biotransformations are of extreme interest to the toxicologist, since in some cases mutagenic or carcinogenic chemicals may be formed. In other instances, chemical reactions may occur which have nothing to do with metabolic processes, but may be significant to health. For example, as mentioned previously, nitrosamines form with extreme ease by the reaction of sec. amines and certain tert. amines with either nitrous acid (HONO) or its various salts. The most important salt is sodium nitrite ($NaNO_2$). However, sodium nitrate ($NaNO_3$) is present in the body to various extents and may be changed to the nitrite form by the action of nitrogen-reducing bacteria. Nitrosamine formation can be catalysed by formaldehyde (HCHO) and thiocyanate ion (SCN^-) and inhibited by ascorbic acid (Vitamin C) and sometimes by dl. tocopherols (Vitamin E). Many nitrosamines are both mutagenic and carcinogenic agents. It is prudent to avoid the use of sec. and tert. amines in aerosol sprays which could be inhaled, since this would act to eliminate any *in vivo* production of possibly dangerous nitrosamines.

During the 1970s, certain groups attempted to place labeling or other sanctions on products containing methylene chloride, stating that the catabolically produced carbon monoxide (CO) reacted quickly with hemoglobin (He) in the blood to produce carboxyhemoglobin (COHe) in dangerous amounts. Investigations showed that levels as high as 20% COHe acted to induce headache and nausea, and could be potentially dangerous for persons with coronary heart disease But such level would never be approached except under the most flagrant conditions of product misuse. Normal levels of COHe range between about 0.5 to 2.0%, but can be increased by smoking or exposure to carbon monoxide from other sources. In a particular test, an aerosol was sprayed into a small, unventilated area to establish a concentration range of 65 to 200 ppm in air, except for a 500 ppm peak, lasting less than 30 seconds. People with pre-test COHe values of 1.1 to 2.1% were exposed to this treated air space, after which their COHe levels changed to a rather uniform 2.1%, indicating an insignificant increase. A study of beauty salon operators working all day in a Time Weighted Average (TWA) of 6.1 ppm methylene chloride showed a 1.4% elevation of COHe over baseline levels.

A consumer aerosol paint remover was used to emptiness in a room with extremely poor ventilation, resulting in a methylene chloride concentration of 80 to 90 ppm in air at the breathing zone and localized peak concentrations of 534 ppm (after 3 minutes) and 698 ppm (after 5 minutes) from the end of the spray period. For safety purposes this type of scenario should be avoided when using such products.

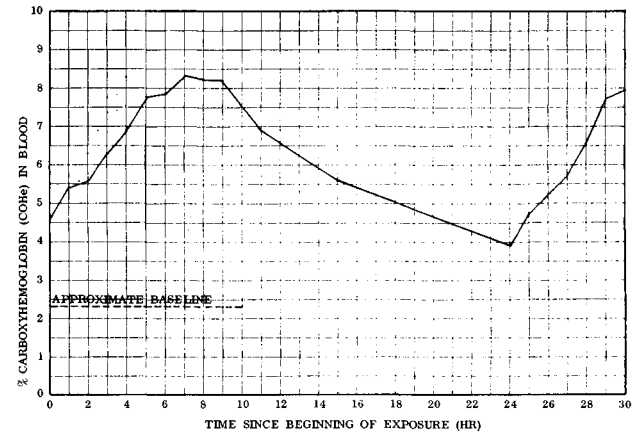


Figure 3. Chart of Exposure to Methylene Chloride
Per cent COH_e in the blood as a function of exposure to a continuous net level of 180 to 200 ppm of methylene chloride during 8-hour workdays. Tests based on an average of seven men (non-smokers).

In one other test, the consecutive use of three hair spray aerosols in a $4' \times 6' \times 8'$ (2.23 m^2) bathroom resulted in the production of an average of 102 ppm of methylene chloride vapors in air across the 15.5 minute period following the last spraying episode. While this situation could be hazardous to some persons, it is strongly magnified from reasonably foreseeable circumstances, where only one can would be sprayed, a larger bathroom would be used, the door would be open, and the person would normally leave the area after spraying the hair. In a recent decision the CPSC determined that no sanctions should be placed on aerosols containing methylene chloride as a result of possible COHe formation in the body.

A final example shows that men exposed to about 190 ppm of methylene chloride vapors continuously in the workplace developed peaks of about 8.35% COHe in the blood, provided they did not smoke. There was about a 74% return to the pre-exposure baseline overnight. These data are elaborated in Figure 3.

Acceptable Limits of Exposure

In the U.S.A. the first formal approach to the control of chemical vapor inhalation in the workplace was taken by the American Conference of Governmental Industrial Hygienists (ACGIH) about 1939. They established the concept of the Threshold Limit Value (TLV) as the maximum to which it was believed workers could be exposed, day after day, without adverse effects. In

1968, these values became legal limits under the Walsh-Healey Act. They were picked up in 1971 by the newly formed Occupational Safety and Health Administration (OSHA), when they adapted a host of industrial consensus standards in writing their regulations.

A term of almost synonymous significance is the Time Weighted Average (TWA) airborne concentration of mists, vapors or gases in the workplace. They are usually numerically identical. They are generally expressed in both ppm or mg/m³ of contaminant, with a maximum level established as 1,000 ppm, except for the special case of carbon dioxide which is higher. TLV or TWA values in one system of measurement can be converted to the other by an easy calculation; for instance a figure of 500 ppm for n-heptane is equivalent to 1,800 mg/m³. TLV figures for various common propellents and solvents are shown in Table V.

TABLE V

Threshold Limit Values (TLV) for Various Aerosol Propellents and Solvents

Vapor in Workplace Air	TLV* (ppm in air)	
P-11	1,000**	
P-12	1,000	
P-22	1,000	
P-113	1,000	
P-114	1,000	
P-115	1,000	
Propane	1,000	
n-Butane	600	
isoButane	800***	
n-Pentane	500	
isoPentane	500	
n-Hexane	500	(100)
n-Heptane	500	(100)
Dimethyl ether (DME)	1,000	
Carbon Dioxide	1,000	
Methylene Chloride - Inhibited	500	(75)
Ethanol	1,000	
Isopropanol	400	
1,1,1-Trichloroethane - Inhibited	500	
Methyl isoButyl Ketone (MIBK)	100	
Diethyl Ether (DEE)	400	
Monoethanolamine	3	
Odorless or Low-odor Kerosene	500	
Toluene	200	
Xylenes	100	

*OSHA values, usually the same as Time Weighted Average (TWA) values.
**Except for the special case of carbon dioxide, the highest TLV or TWA recommendations are 1,000 ppm. or 0.1v%.
***isoButane is listed but no value is specified. 800 ppm is an estimate.
Values in parentheses are those of the National Institute of Safety and Health, indicated when different from the TLVs. Other values, such as those by the EEC for MAC, and those currently proposed by the ACGIH, may be even lower.

The National Institute of Safety and Health (NIOSH) functions in an advisory capacity to such agencies as OSHA and the EPA, and they frequently propose revisions in the TLV/TWA values in accordance with current clinical findings and consensus opinions.

For instance, they have now asked for a reduction to 50 ppm in the case of n-hexane (consistant with the European MAC value) which recognizes such recent findings as (1) 100 ppm can cause neuropathy in chickens after 4 to 5 weeks of continuous exposure, (2) 250 ppm causes mild neuropathy in the mouse after 6 d/wk for one year and (3) workers exposed to 500-1000 ppm 8 hr/d showed impaired sensory perception, loss of strength, muscular atrophy and other factors that continued for up to one year after exposure was removed. The proposed level of 50 ppm would provide a safety factor of approximately ten-fold.

Many TLV/TWA listing include an extra allowance for short term exposures. Although both n-hexane and n-heptane are listed as 500 ppm for 8 hour exposure averages, concentrations of 1,800 ppm are allowed for periods to 15 minutes. In the case of ethylene glycol monomethyl ether (EGMME), $CH_3O.CH_2.CH_2OH$, the OSHA TWA is 25 ppm, while the ACGIH has a Permissible Exposure Limit (PEL) of 25 ppm 8-hr TWA and a Short Term Exposure Limit (STEL) of 35 ppm for any 15 minute excursion. Those wishing to determine the TWA value for particular chemicals may consult the U.S. *Federal Register 29* Sec. 1910.1000 in the OSHA regulations.

Material Safety Data Sheets

An easier way to determine TWAs plus a great deal of other toxicological information is to refer to a Material Safety Data Sheet, OSHA Form 20, or forms essentially similar, which have been approved by the U.S. Department of Labor. An example of a properly filled out form is shown as Table VI (Pages 263, 264), except for the deletion of the manufacturer and relevant data.

Around 1974 the aerosol industry recognized a need for such a form for aerosol products. A special task force under the aegis of the CSMA Aerosol Division developed a "Material Safety Data Sheet for Pressurized Products" during the following year and had it approved by the government as one essentially similar to the OSHA-20 form. This two-page form is shown as Table VII (Pages 265, 266).

(continued on page 267)

TABLE VI

MATERIAL SAFETY DATA SHEET

(Approved by U.S. Department of Labor "Essentially Similar" to Form LSB-00S-4)

CHEMICAL NAME: ETHANOL, 200 PROOF

SYNONYMS: Ethyl Alcohol, 200 Proof — CHEMICAL FAMILY: Alcohols

FORMULA: C_2H_5OH — MOLECULAR WEIGHT: 46.07

TRADE NAME AND SYNONYMS: Ethanol, 200 Proof; Ethyl Alcohol

I. PHYSICAL DATA

BOILING POINT, 760 mm. Hg	78.3°C. (172.9°F.)	FREEZING POINT	-114.1°C.
SPECIFIC GRAVITY ($H_2O = 1$)	0.7905 at 20/20°C.	VAROR PRESSURE at 20°C.	44 mm. Hg
VAPOR DENSITY (air = 1)	1.6 at 78.3°C.	SOLUBILITY IN WATER, % by wt. at 20°C.	Complete
PER CENT VOLATILES BY VOLUME	100	EVAPORATION RATE (Butyl Acetate = 1)	3.30
APPEARANCE AND ODOR	Water-white liquid; characteristic odor.		

II. HAZARDOUS INGREDIENTS

MATERIAL	%	TLV (Units)
Not applicable		

III. FIRE AND EXPLOSION HAZARD DATA

FLASH POINT (test method)	70°F., Tag open cup	AUTOIGNITION TEMPERATURE		793°F.	
FLAMMABLE LIMITS IN AIR, % by volume		LOWER	4.3	UPPER	19.0

EXTINGUISHING MEDIA	Carbon dioxide or dry chemical for small fires. "Alcohol"-type foam for large fires.
SPECIAL FIRE FIGHTING PROCEDURES	Addition of water may reduce intensity of the flames.
UNUSUAL FIRE AND EXPLOSION HAZARDS	None

EMERGENCY PHONE NUMBERS

John D. Doe 217-443-1400
Jane D. Buck 217-444-1400

Legal responsibility is assumed only for the fact that all studies reported here and all opinions are those of qualified experts.

ABC Corporation, 555 Western Avenue, Chicago, IL 60699

TABLE VI - *Continued*
Side 2 of Material Safety Data Form

IV. HEALTH HAZARD DATA

THRESHOLD LIMIT VALUE	1,000 ppm.
EFFECTS OF OVEREXPOSURE	Swallowing liquid causes inebriation, headache, nausea, and vomiting. Liquid causes eye irritation. Breathing of vapors may cause drowsiness.
EMERGENCY AND FIRST AID PROCEDURES	Flush skin and eye contact with plenty of water. If inhaled, remove to fresh air; give artificial respiration if breathing has stopped. Call a physician. If swallowed, induce vomiting.

V. REACTIVITY DATA

STABILITY			
UNSTABLE	STABLE	CONDITIONS TO AVOID	Avoid heat, sparks, and fire.
--	√		

INCOMPATIBILITY (materials to avoid)	None
HAZARDOUS DECOMPOSITION PRODUCTS	Thermal decomposition may produce carbon monoxide and/or carbon dioxide.

HAZARDOUS POLYMERIZATION			
May Occur	Will not Occur	CONDITIONS TO AVOID	None
--	√		

VI. SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED	Flush heavily with water.
WASTE DISPOSAL METHOD	Incinerate in a furnace.

VII. SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION (specify type)		None required			
VENTILATION	LOCAL EXHAUST	Preferable	SPECIAL	--	
	MECHANICAL (general)	Acceptable	OTHER	--	
PROTECTIVE GLOVES		None required	EYE PROTECTION	Goggles	
OTHER PROTECTIVE EQUIPMENT		Eye bath and safety shower			

VIII. SPECIAL PRECAUTIONS

PRECAUTIONARY LABELING	ETHANOL, 200 PROOF On the basis of the toxicological, physical, and chemical properties of ETHANOL, 200 Proof, precautionary labeling used on the containers is as follows: FOR INDUSTRY USE ONLY
OTHER HANDLING AND STORAGE CONDITIONS	--

Printed in U.S.A.

TABLE VII

MATERIAL SAFETY DATA SHEET FOR PRESSURIZED PRODUCTS

PRODUCT TRADE NAME:

PRODUCT DESCRIPTION:

SECTION I - PRODUCT IDENTIFICATION	
COMPANY NAME	Regular Telephone No. Emergency Telephone No.
ADDRESS	
PRODUCT TRADE NAME OR BRAND NAME	
OTHER PRODUCT DESCRIPTION OR IDENTIFICATION	

SECTION II - HAZARDOUS INGREDIENTS				
INGREDIENT CHEMICAL NAME	TYPE OF HAZARD(S)	APPROXIMATE WEIGHT %	TLV VALUE	OTHER TOXICITY INFORMATION

SECTION III - PHYSICAL DATA			
BOILING POINT @ 101.3 kPa (760mm Hg)	◯Not Applicable °C (°F)	SPECIFIC GRAVITY/DENSITY (vs. water @4°C/39.2°F)	◯Not Applicable Mg/m³
VAPOR PRESSURE MAXIMUM @ 54.5°C (130°F)	kPa (psig)	PERCENT VOLATILES (Ambient/21°C/70°F)	Approximately % by Volume
VAPOR DENSITY vs. AIR=1 @15-32°C (60-90°F)	◯Heavier= >1.0 ◯Lighter= <1.0	EVAPORATION RATE (vs. n-Butyl Acetate=1)	◯Not Applicable ◯ <1.0 ( ) >1.0
SOLUBILITY IN DEIONIZED WATER (Weight % @10°C/50°F)	◯Negligible= <.1% ◯Slight= .1-1.0%	◯Moderate= 1-10% ◯Appreciable= >10%	◯Complete % by Wt.
APPEARANCE AND ODOR (Description of spray and resultant residue)			

SECTION IV - FIRE AND EXPLOSION HAZARD DATA			
FLASH POINT (minimum) Method:	◯Not Applicable °C (°F)	FLAMMABLE LIMITS IN AIR (Vol. % @ °C/ °F)	◯Not Applicable LEL-UEL= %
EXTINGUISHING MEDIA: ◯Not Applicable (will not burn below 815°C/1,500°F) ◯Water Fog ◯Standard Foam ◯Special Alcohol-Stable Foam ◯Carbon Dioxide-CO_2 ◯Dry Chemical ◯Special:			
SPECIAL FIREFIGHTING PROCEDURES: Keep containers cool. Use equipment or shielding required to protect personnel against bursting, rupturing, or venting containers.			
UNUSUAL FIRE AND EXPLOSION HAZARDS: At elevated temperatures (over 54°C/130°F), containers may vent, rupture, or burst. Also see Section VI.			

**Additional Notes or Comments from sections above:

(Continued on Side 2)

Date Filled Out: ; Prepared or Approved by:

THIS FORM APPROVED BY THE **U.S. DEPARTMENT OF LABOR** AS "ESSENTIALLY SIMILAR" TO **OSHA-20.**

Unless noted otherwise, all information given is on the total product, including propellents.

Form L7521IA LCM

TABLE VII* - *Continued*

Side 2

MATERIAL SAFETY DATA SHEET FOR PRESSURIZED PRODUCTS (Continued)

PRODUCT TRADE NAME:

SECTION V - HEALTH HAZARD DATA

THRESHOLD LIMIT VALUE (TLV): ( )Not Applicable ( ) ppm ( )

EFFECTS OF OVEREXPOSURE:

EMERGENCY AND FIRST AID PROCEDURES: If unconscious, remove victim to fresh air and call a physician. If gotten in eyes, flush immediately with large amounts of water. ( )Contains cardial sensitizer - if unconscious from inhalation, do not give adrenalin-type drugs. ( )

SECTION VI - REACTIVITY DATA

CHEMICAL STABILITY: ( )STABLE ( )UNSTABLE - CONDITIONS TO AVOID:

INCOMPATABILITY (Materials to avoid): ( )None with common materials ( )

HAZARDOUS DECOMPOSITION PRODUCTS (From burning, welding, oxidation, high temperatures): ( )None ( )Carbon Monoxide ( )Phosgene ( )Hydrofluoric Acid ( )Hydrochloric Acid ( )

HAZARDOUS POLYMERIZATION: ( )Will not occur ( )May Occur - Conditions to avoid:

SECTION VII - LEAK AND DISPOSAL PROCEDURES

STEPS TO BE TAKEN IF CONTAINERS ARE LEAKING OR LARGE AMOUNTS ARE RELEASED: ( )Avoid breathing vapors. ( )Remove ignition sources. ( )Avoid skin contact with liquid. ( )

WASTE DISPOSAL METHOD: Do not puncture or incinerate containers. Give empty, leaking, or full containers to a disposal service equipped to safely handle and dispose of pressurized containers.

SECTION VIII - SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION (Specify type): ( )Not Applicable. ( )

VENTILATION | LOCAL EXHAUST (Hoods, Fans, etc.): ( )Not Applicable. ( ) | SPECIAL: ( )Not Applicable ( )

MECHANICAL (General Area Ventilation): ( )Not Required ( )Should be on while spraying, to remove solvent vapor.

PROTECTIVE GLOVES: ( )Not Applicable Wear: ( )Rubber ( )Vinyl ( )Polyethylene ( ) gloves while spraying.

EYE PROTECTION: ( )Not Applicable ( )Wear Goggles while spraying. ( )

OTHER PROTECTIVE EQUIPMENT: ( )Not Applicable ( )Do not wear clothing soaked by spray. ( )

SECTION IX - SPECIAL PRECAUTIONS

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE: Do not store where temperatures could exceed ( )54°C (130°F) ( ) °C (°F). ( )

OTHER PRECAUTIONS:

SPECIAL PRECAUTIONARY STATEMENTS: **Please read and follow the directions on the product label; they are your best guide to using this product in the most effective way, and give the necessary safety precautions to protect your health.**

PRODUCT TRADE NAME:

PRODUCT DESCRIPTION:

Form L751211B LCM

**Additional Notes or Comments from sections above:

Date Filled Out: ; Prepared or Approved by:

The accuracy of data and information given on this form is not guaranteed, but it has been filled out to the best of our knowledge and belief. If you find any errors or have any suggestions to improve the presentation, please contact us at the address on the first sheet.

*Table VII appearing on pages 265, 266 is a reproduction of the two sides of Form L751211A LCM, the Material Data Sheet for Pressurized Products

Marketers are increasingly requiring the submission of OSHA-20 type forms from chemical suppliers, for individual components, and fillers, for total formulations. In addition, it is prudent to have a file of these forms available in case of chemically related plant accident, so that proper measures can be taken, even by relatively untrained personnel.

The toxicological properties of a formulation are not normally the same as those predicted by taking a weighted average of the properties of the ingredients. For assurance they must be determined by testing the total formulation. The tests described under CPSC/FHSA and EPA/FIFRA have been mentioned. Similar tests are used under FDA and TSCA programs. In some cases, these procedures are over twenty years old and outdated. During 1980/1981 the Cosmetic, Toiletries and Fragrance Association (CTFA) Pharmacology and Toxicology Committee developed a series of nine safety testing guidelines for evaluation of various types of toxicity and irritation/sensitization. It is hoped that the regulatory procedures will be reevaluated and modified accordingly. These guidelines have now been published by the CTFA.

Considering the acute tests described in the regulatory literature and mentioned earlier, the 1981 costs for having them performed at a typical outside laboratory are indicated in Table VIII.

Other acute tests may be performed under special circumstances, such as a photosensitization study, vaginal irritancy test and teratology assay. They are not always relatively inexpensive; the teratology assay cost from $17,500 to $22,000 in 1981.

Sub-acute Toxicity

In toxicology the term "sub-acute" (or, sometimes, "sub-chronic") suggests a study of intermediate duration, where exposure lasts for a period of several days to a few months. A typical sub-acute inhalation or sub-acute feeding study would be conducted for 90 days. The objectives are to gain more specific information about the biological response to repeated exposures or multiple doses of a substance. These tests are used frequently to determine optimum protocols for chronic tests of still longer duration and greater expense.

Although sub-acute tests almost never last more than six months, such a study would cover 25% of the average life span of the rat. Or it would be the equivalent of 202 months (17 years) in man. So, when done with certain animals, there are very definite chronic implications. Despite the time-related standards developed by the World Health Organization (WHO) in 1966 where the acute, sub-acute or chronic definition was established with respect to duration administration, many toxicologists feel that the percentage of the life span of the test animal should be the controlling factor. The WHO standards are now very controversial, because of this and some other considerations.

The term sub-chronic is used increasingly to describe tests where the administrative phase is from about 3 to 12 months duration. The WHO standard limits sub-acute studies to three months, and on the other hand, true chronic studies usually take from two years to the lifetime of the test animals. A comparison of the five time-related divisions of toxicological testing is given in Figure 4.

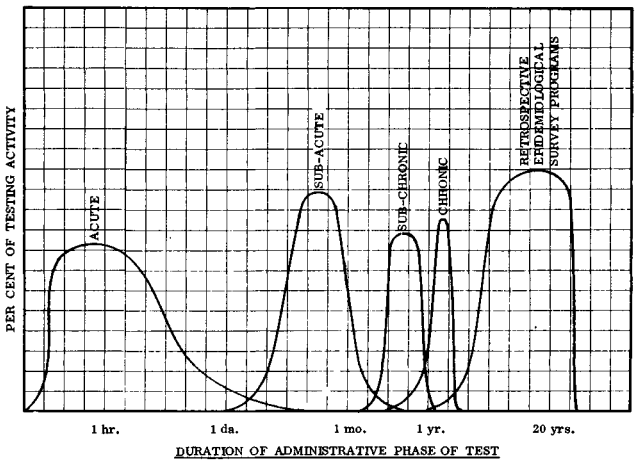


Figure 4. Toxicological Test Program Chart
Arbitrary time-related divisions of toxicological testing program (approximate).

TABLE VIII

Fee Schedule (1981) for Acute Toxicology Studies
(Average pricing)

Acute Oral Toxicity	10 rats	$190
Acute Inhalation Toxicity	10 rats	600
Acute Dermal Toxicity	10 rabbits	600
Primary Skin Irritancy*	6 rabbits	260
Primary Eye Irritancy*	6 rabbits	260
Dermal Sensitization Assay**		
Dose Range Finding	4 guinea pigs	250
Landsteiner Method	10 guinea pigs	1,200

*Draize Method.
**Not normally performed unless a known strong sensitizer is present.
Reference: *Federal Register*, CPSC 1500.3

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