2014 LECTURE NOTES
Inhalation Toxicology and Toxic Responses of the Lung

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BIOGRAPHICAL SKETCH

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BIOGRAPHICAL SKETCH

Yves Alarie was a Professor of Respiratory Physiology and Toxicology in the Environmental and Occupational Health Department of the Graduate School of Public Health, University of Pittsburgh. Prior to joining the University in 1970 and retiring in 2002, he held various positions in the Inhalation Division at Hazleton Laboratories starting in 1963, after graduating from the Université de Montréal. His research interests are focused on the effects of airborne chemicals at the surface of the respiratory tract, from the tip of the nose to the alveolar level. He has published extensively on the use of animal models to estimate safe levels of exposures for airborne chemicals of industrial importance, as well as their mixtures. He also conducted extensive research on the toxicity of smoke produced in fires and the principal causes of death in fire victims.

TOPICS COVERED IN THIS LECTURE

1. Description of contaminants
2. Concentration and volume units
3. Other concentration units
4. Concentration units for aerosols
5. Acute inhalation toxicology
6. Exposure chambers
7. Exposure systems for calculation of dose
8. Factors influencing the dose for aerosol
9. New nomenclature
10. Characteristics of aerosols influencing their toxicity

TOPICS COVERED IN THIS LECTURE

11. Retention and clearance of aerosols
12. Factors influencing the dose for gases and vapors
13. Effects on the respiratory tract
14. How to evaluate sensory irritation
15. Pulmonary sensitizers
16. Chronic effects
17. Standards for exposure to airborne contaminants
18. Recent occupational pulmonary disease
19. Uptake of carbon monoxide

CHAPTER 1: DESCRIPTION OF CONTAMINANTS

A. *GAS*
A state of matter in which the molecules are practically unrestricted by cohesive forces. A gas has neither shape nor volume for our use. It is a substance which has a critical temperature below 200C and thus cannot be condensed into liquid form at any pressure at this temperature.

Examples with their critical temperatures in parentheses: Methane (-82^{o}C), Fluorine (-129^{o}C), Helium (-268^{o}C)

1-1

B. *VAPOR*

Substances dispersed in air as individual molecules, below their critical temperature and thus could be condensed to a liquid at 20°C by increasing the pressure. A vapor has neither shape nor volume for our use.

Examples: Iodine (512°C), Benzene (289°C), Carbon Disulfide (279°C)

Therefore, the words "vapor" and "gas" are often used interchangeably. Vapor is more frequently used for a substance which, although present in the gaseous phase at 20°C, exists as a liquid or solid at this temperature and normal atmospheric pressure. We can also say that SO_2 (157°C), Cl2 (144°C), NO_2 (158°C), and CO_2 (289°C) can be obtained as "vapor" since at 20°C they can be condensed as a liquid by increasing the pressure. Their critical temperature (given in parenthesis) is above 20°C. However, since they are normally purchased in steel cylinders under pressure we usually refer to them as gases.

1-2

C. *AEROSOL*

Stable or quasi-stable suspension of solid or liquid particles in a gas. Various terms are used to define an aerosol on the basis of its origin or state. These terms are:

- **> Fumes**
- **> Dusts**
- **> Mists**
- **> Fog**
- **> Smoke**
- **> Haze**
- **> Smog**
- **> Thermal Decomposition Products (TDP)**

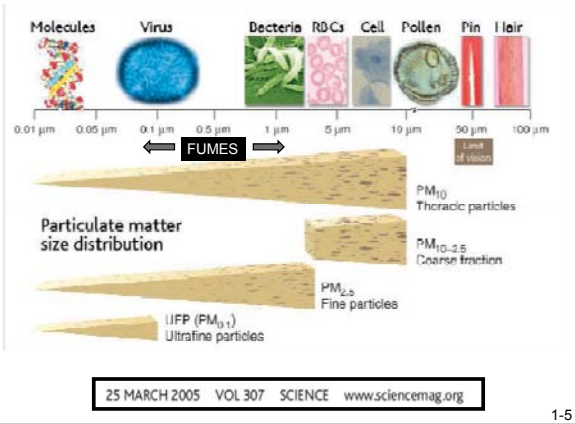
1-3

FUMES:

Solid particles formed by condensation. Generally used for metals such as Cd, Pb, Zn, etc., but has also been used for any solid, after heat treatment, such as Teflon or PVC fumes.

Strictly speaking, Teflon or PVC are not fumes since the particles are made of liquids, solids and dissolved gases. The appropriate term is "smoke" or "thermal decomposition products" as given below. Usually well below 1 µm in diameter and fairly homogenous, in the case of metals.

1-4



1-5

DUSTS:

Solid particles formed during disintegration processes of a mechanical nature; mining, grinding, (i.e., coal dust), etc. Usually above 1 µm in diameter and heterogeneous, less stable because of larger size and polydispersed.

MISTS:

Refers to liquid particles, formed by condensation of a vapor (small particles, homogenous, stable) or by atomization of a liquid (larger particles and less stable).

FOG:

A mist which appreciably reduces visibility.

1-6

SMOKE:

Particles in suspension in air resulting from flaming combustion or pyrolysis of organic or inorganic materials. The particles may contain solids, liquids and dissolved gases. The particles can be very small or very large depending upon a variety of conditions.

HAZE:

A combination of vapors, dusts, fumes, mists or smokes which appreciably reduces visibility.

Sometimes haze is used instead of fog (i.e., hazy vs. foggy) to describe reduced visibility. If water vapor condensation is the main culprit, foggy should be used instead of hazy.

1-7

SMOG:
Older definition was for the combination of smoke and fog as in the London smog which resulted in 4,000 deaths in 1952. More recently, used in the U.S. to describe the resulting combination of gases and aerosols formed during U-V irradiation of hydrocarbons, oxides of nitrogen and oxygen and the resulting ozone, etc. (i.e., L.A. smog). This will occur where a large amount of automobile exhaust and sunlight are present under atmospheric conditions unfavorable to dispersion of pollutants (Magill, 1949). Chemical reactions involved were given by Haagen-Smith in 1955. A better term is "photochemical smog".

From: Magill, P L (1949). The Los Angeles Smog Problem. Indust. Engin. Chem. 41: 2476-2486.

1-8

PITTSBURGH 1944

… AT NOON

http://arllib2.msha.gov/awweb/main.jsp?flag=browse&smd=1&awdid=18
http://arllib2.msha.gov/awweb/main.jsp?flag=browse&smd=1&awdid=29

1-9

THERMAL DECOMPOSITION PRODUCTS (TDP):
Combination of vapors, liquids and solids formed from synthetic polymers (plastics) during operations such as molding, cutting with hot wires, heat treatment of any kind.

1-10

D. *OTHER TERMS TO DESCRIBE AEROSOLS*
Aerosols are also described according to their chemical composition or their size distribution by the following terms:

- **HOMOGENEOUS:**
  Refers to chemical constitution (i.e., sulfuric acid)
- **HETEROGENEOUS:**
  Refers to chemical constitution, (i.e., coal dust)

1-11

MONODISPERSED:
Refers to distribution of particle sizes around the geometric mean or median. Usually when the geometric standard deviation (abbreviated σ_g) is 1.2 or less, an aerosol is said to be Monodispersed (i.e., all the particles, or nearly so, are of the same size). Obviously we don't have this in industrial exposures.

POLYDISPERSED:
As above, geometric standard deviation larger than 1.2. The term, "heterodispersed", is also used.

1-12

CHAPTER 2:
CONTRATION AND VOLUME UNITS

A. *mg/m³*
Milligrams of pollutant per cubic meter of air. Can be used for a gas, vapor or aerosol. Always correct to use. You will also encounter µg/L. Now with SI nomenclature, you may encounter g/m³.

Please do not use g/m³, nonsense.

Note: When you want to compare the potency of airborne chemicals on a molar basis, simply divide mg by molecular weight to obtain millimole/m³ or micromole/L, just like you can use millimole/kg of body weight instead of mg/kg of body weight. When you have the concentration in ppm, as below, this conversion is already made; plus a constant as given below.

2-1

B. *ppm*

Parts per million = volume of gas or vapor to volume of air relationship (i.e., one mL of benzene *vapor* (not one mL of benzene liquid) in 1,000,000 mL of air. Since we use a volume of gas or vapor, this unit cannot be used for aerosols.

$$\frac{\text{Volume of Vapor or Gas Pollutant} \times 10^6}{\text{Total Volume of Container or Total Volume of Air Sample}}$$

2-2

C. *VOLUME PERCENT*

Same volume/volume relationship as ppm. Used for high concentrations (i.e., 1% or 0.1% instead of 10,000 or 1,000 ppm respectively).

D. *mg/m³ to ppm*

(mg/m³ x 24. 5) / MW = ppm, MW= Molecular Weight

Note: 24.5 is used for 20-25°C, while 22.4 is used for 0°C. These values are simply the volume (liter) occupied by one mole (gram/MW) of a chemical at a given temperature and sea level atmospheric pressure (i.e., 760 mm Hg).

E. *ppm to mg/m³*

(ppm x MW) /24.5 = mg/m³, MW = Molecular Weight

2-3

F. *fibers/cc (of air)*

Number of fibers per cubic centimeter

Example: asbestos

G. *mppcf or mp/ft³ (of air)*

Million of particles per cubic foot* *Note: 1ft³ = 28.32 liters

Example: silica

Note: Number of fibers or particles in a volume of air was used earlier because it was more accurate to sample a volume of air by passing it through a membrane filter and then counting the particles present on the filter by using a microscope. With electronic balances available today it is much easier to weigh the filter before and after air sampling and get the difference in mg. Knowing the mass deposited on the filter and the volume of air sampled through the filter gives the answer in mg/m3. This is called "gravimetric" analysis. The other way is to perform a chemical analysis for the aerosol collected on the filter. However, there are still exceptions (i.e., asbestos, requiring microscopic evaluation).

H. *#/cm³ (of air)*

Number per cubic centimeter

Example: nanoparticles

2-4

H. *INDUSTRIAL AND TOXICOLOGICAL APPLICATIONS*

● Dilution to TLV® or PEL or NAQS

It is important to understand that when very low concentrations are needed for protection against a toxic effect, a small amount of a gas or vapor will need a lot of air dilution. It is not normally possible to rely on air dilution alone in these instances. Engineering controls such as hoods, etc. must be instituted. This is also happening in Los Angeles. Every time a gasoline tank is filled, gasoline vapors are produced. Since the level of hydrocarbons must be maintained very low to avoid formation of photochemical smog and ozone, they have no choice but to capture these vapors, they no longer can rely on simple air dilution.

2-5

● Dilution to TLV® or PEL

Assume 1 mL (~1,000 mg) of toluene diisocyanate (TDI) evaporates completely. The 2014 ACGIH Threshold Limit Value (TLV®-TWA) is 0.005 ppm or 0.035 mg/m³.

- **For dilution to 1 ppm (~7 mg/m³): need 142 m³ of air**
- **For dilution to 0.005 ppm: need 28,400 m³ of air**

  Equivalent to a building of

  60 x 30 x 450 feet

2-6

● Disasters Even With Huge Dilution

A large amount, even with huge air volume dilution, goes a long way. The episode in Bhopal in 1984 illustrates the point. With 10-25 tons of evaporating and reacting methyl isocyanate (MIC), even with large air dilution, the results were devastating; the most serious industrial accident. The LC50 for MIC (4 hours exposure and following victims for 7 days) for humans is probably around 10-30 ppm (a best guess from animal studies) and, therefore, when released in a populated area, MIC resulted in at least 5,000 deaths and 100,000 injured individuals. Of these, probably 5,000-10,000 have permanent pulmonary injuries. Therefore, storage of large quantities can present a serious hazard.

2-7

● **Saturation Concentration (Cs)**
This is the maximum amount that can exist as vapor above a liquid, or "saturation concentration". It can be estimated:

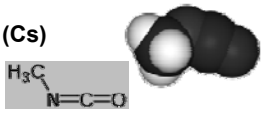
$$Cs = (MW \times T \times p \times 10^6) / (22.4 \times 273 \times BP) = mg/m^3$$

p = Vapor pressure (mm Hg)
T = Temperature (K)
BP = Barometric Pressure (mm Hg)
22.4 = Volume (liter) occupied by 1 mole of gas at 0°C (273°K) and 760 mm Hg (use 24.5 at 22°C)

One should know Cs for any liquid and while it can never be achieved in open air (probably around 20-25% of it, unless right above the spill) Cs should then be compared to toxic levels such as the LC50, or to safe levels such as the TLV®.

2-8

● **Saturation Concentration (Cs)**
Example: MIC



Cs for MIC = 350,000 ppm
Since the 4-hour LC50 with deaths observed within 7 days is only around 10 to 30 ppm, it is obvious that if there is a spill, the situation will become very hazardous very quickly. The vapor pressure of a chemical is therefore very important in assessing the *hazard* of a spill since it is the primary factor in determining how high the concentration in the air can be. The toxic *hazard* of chemicals is not only due to their *potency* but also due to their *vapor pressure*.

2-9

END-NOTE

Toxicologists seldom take into account the vapor pressure of chemicals in their evaluation of toxic hazard. They only look at potency (i.e., how much to produce a given effect). From this, lists of "most hazardous" chemicals are produced. **This is NONSENSE.** They will go one step further, multiplying the potency by how many pounds are produced/year. **MORE NONSENSE.** And even further, by multiplying by the number of individuals potentially exposed. Fortunately, such practices are going away. In many industrial operations, heat is involved. Thus, chemicals with very low vapor pressure can volatilize and then condense as aerosols when entering cooler air. We then have two phases: vapor and aerosol. Much like a fog containing both water vapor and water aerosol. An article on the influence of vapor pressure for proper inhalation toxicology practice should be consulted.

Perez, C and Solderholm, SC (1991). Some chemicals requiring special consideration when deciding whether to sample the particle, vapor or both phases of an atmosphere. Appl. Occ. Environ. Hyg. 6: 859-864.

2-10

CHAPTER 3:
OTHER CONCENTRATION UNITS

A. *PARTIAL PRESSURE*
Fraction (Volume/Volume Composition) of the total pressure of a mixture exerted by a component of the mixture. For example, at sea level, 1 atmosphere (760 mm Hg, 760 torr, 1.1013 bars, 1.03 kg/cm^2, 14.7 p.s.i.a. or 101.325 kPa and yes you were under the impression that SI units were going to simplify your life, fat chance!), if we know the volume composition of each component in the mixture, we can calculate the partial pressure of each.

3-1

Assuming the following % composition for air:

N_2	**= 78.6% = 597 mm Hg Partial Pressure**
O_2	**= 20.8% = 159 mm Hg Partial Pressure**
CO_2	**= 0.04% = 0.3 mm Hg Partial Pressure**
H_2O	**= 0.5% = 3.8 mm Hg Partial Pressure**
TOTAL	**100% = 760 mm Hg Total Pressure**

The volume composition can be in % or ppm (see above), since both are volume/volume unit. Therefore, 0.1% or 1,000 ppm = 0.76 mm Hg. From the above, there is a very easy way to calculate Cs in ppm when you know the vapor pressure in mm Hg at 22-25°C, since 760 mm Hg = 1,000,000 ppm. For TDI with a vapor pressure of .02 mm Hg, the answer is: 1,000,000/760 × .02 = 26 ppm.

3-2

B. *pV = nRT*
If the concentration is given as moles/Liter (n/V), the partial pressure p (in mm Hg) can be obtained using the value of the gas constant R as 62 and the temperature (T) in Kelvin. Seldom used in toxicology, but certainly appropriate.

C. *HENRY'S LAW AND SOLUBILITY COEFFICIENT*
The amount (moles or grams) of a gas or vapor dissolved in a liquid is directly proportional to the partial pressure of the gas in the gas (air) phase above the liquid. At equilibrium, the partial pressure (in mm Hg) in both phases is equal, but the amount, in moles or grams, will vary between the two phases according to each gas and the nature of the liquid.

3-3

This ratio, S, is called the solubility coefficient and is given at particular temperature.

S = Concentration in moles (or grams) of a gas in a liquid/ concentration (same units) of a gas in air phase

S = (C liquid) / (C air or C gas)

Table 1. Distribution or partition (Solubility Coefficient) for a Gas at Equilibrium Between Liquid and Air Phases[a]

Phases	Partial Pressure (mm Hg)	Concentration (mg/L)	Solubility Coefficient
Air or Gas	100	50	100
Liquid	100	5,000	

[a]At equilibrium the partial pressure is equal in both phases but not the concentration in mg/L, unless S = 1.

Note: Sometimes S is given as C gas/C liquid; please check this when consulting a textbook. Also referred to as the Ostwald solubility coefficient and sometimes abbreviated L instead of S.

3-4

Table 2. Some Examples of Solubility Coefficients

Cl/Cg = 1/100 = 0.01 = Low: Nitrogen, Helium, Oxygen

Cl/Cg = 150/100 = 0.15 = Moderate: CO_2, N_2O

Cl/Cg = 50/100 = 0.5 = Moderate: Vinyl Ether

Cl/Cg = 1500/100 = 15 = High: Diethyl Ether

Cl/Cg = 110000/100 = 1,100 = Very high: Ethanol

Liquid = Water, T = 37^{O}C

3-5

Note: Water/Gas and Blood/Gas S values are usually close. However sometimes (as below) the Blood/Gas S values can be much higher than Water/Gas. This will occur when protein(s) in blood has a high affinity for the substance. A good example is carbon disulfide for which hemoglobin has a high affinity. The best examples are oxygen and carbon monoxide for which hemoglobin has a very high affinity. Thus one must be careful about S values for Water/Gas; however they are a good starting point if S value for Blood/Gas is not known. S values for Blood/Gas may also vary from species to species.

Note: Two articles should be consulted on partition coefficients for nonreactive volatile organics.
Poulin, P and Krishnan, K (1996). A tissue composition-based algorithm for predicting tissue: air partition coefficients of organic chemicals. Toxicol. Appl. Pharmacol. 136, 126-130.

Poulin, P and Krishnan, K (1996). A mechanistic algorithm for predicting blood: air partition coefficients of organic chemicals with the consideration of reversible binding in hemoglobin. Toxicol. Appl. Pharmacol. 136, 131-137.

Note: Another problem with affinity for proteins is that this process is saturable and therefore S may vary with concentration. Again oxygen and carbon monoxide are good examples of this phenomenon.

3-6

Table 3 and Table 4. Examples of Solubility Coefficients For Industrial Chemicals

Table 3: Substances	Water/Gas	Oil/Gas	Blood/Gas	Species
Acetone	395	86	245	Man
Benzene	2.8	492	7.8	Man
Carbon tetrachloride	0.25	361	2.4	Man
Cyclopropane	0.21	11.5	0.55	Man
Methyl Ethyl Ketone	254	263	201	Man
Sulfur Hexafluoride			0.006	Man

Table 4: Substances[a]	Saline/Gas	Blood/Gas
1,1-Dichloroethylene	0.4	5
Bromochloromethane	8	41
Diethyl Ether	11	12
Methyl Chloroform	0.75	5.78

[a]From Gargas and Andersen, 1985

3-7

CHAPTER 4: CONCENTRATION UNITS FOR AEROSOLS

A. *MASS CONCENTRATION*

mg/m^3 for an individual substance or a mixture of substances (i.e., sulfuric acid, coal dust). This refers to the amount (mass) of the substance (mg) dispersed in one cubic meter of air (m^3).

Explosion of 700 pounds of coal dust issuing from main entry of experimental coal mine. Bruceton, PA. (Saturday, April 26, 1947)

http://arllib2.msha.gov/awweb/main.jsp?flag=browse&smd=1&awdid=27

4-1

B. *SIZE CONCENTRATIONS*

Distribution, physical size, vs. mass size

If a sample of an aerosol is taken it is very unlikely that the size will be the same for all particles. In practice it has been found that the size distribution is best represented by a log normal distribution and from this, the median size is obtained and the standard deviation is also obtained. Since this is a log-normal distribution the standard deviation is the geometric standard deviation, abbreviated σ_g. The distribution can be for the physical size (i.e., count median diameter, CMD) or for the mass size (i.e., mass median diameter, MMD).

4-2

1. Physical Size, Count Mean Diameter (CMD)
This is obtained by sampling the aerosol on a filter or appropriate media and "sizing" the collected particles under a light microscope or electron microscope. Also a variety of other methods are available using laser, etc.

2. Mass Size, Mass Median Diameter (MMD)
Most commonly, it is obtained experimentally by using an impactor which has been previously calibrated using spherical particles of a chemical of unit density. When this is done, the shapes as well as the density of the particle being measured are taken into account.

4-3

Since both shape and density are taken into account this can be called "mass median aerodynamic diameter" (MMAD), or "mass median aerodynamic equivalent diameter" (MMAED) instead of simply MMD.

The concept here is: if a particle of a given shape and given density impacts where a 1 μm spherical particle of unit density impacts, it is said to "behave aerodynamically" as a 1 μm spherical of unit density particle behaves.

4-4

Since particle penetration and deposition in the respiratory tract is due to two factors, impaction and sedimentation, and for both mechanisms of deposition the size, shape and density of the particle are primary factors it follows that MMAD must be obtained. This is true for particles > 0.5 μm. For particles < 0.5 μm, a third factor is important: diffusion or Brownian movement. For these particles, their diffusion coefficient is important and this varies with their size, but is independent of their shape or density (i.e., they behave like gas molecules). We will discuss this again below.

4-5

C. *AERODYNAMIC SIZE: DIRECT MEASUREMENT*
This can be measured experimentally by using a calibrated impactor. The mass of particles deposited on each stage of the impactor is obtained and distribution by mass is then derived. A variety of impactors are available. One is described in the next slide.

1. **You get the particle size distribution and from this, you can determine where the particles are likely to be deposited in the respiratory tract.**
2. **You get the exposure concentration (mg/m^3) by adding the mass deposited on each stage of the impactor and on the last stage which is a filter collecting the particles too small to be captured by any stage of the impactor.**

4-6

Marple Series 290 Personal Cascade Impactor

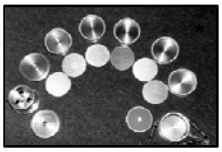
IMPACTOR OPERATING PROCEDURE:

Prior to sampling, the collection substrates and back-up filter are pre-weighed and placed in the impactor. The sampling flow rate of the personal sampling pump is set at 2 LPM. The personal mounting bracket is attached to the lapel or pocket. After sampling, the impactor is disassembled and the substrates and filter weighed. If desired, the samples are analyzed chemically. The weight increase on each substrate is the mass of particles in the size range of that impactor stage. The total weight of particles on all stages and filter is added up, and the percent particle mass in each size range is calculated. Usually, the data is presented graphically as a cumulative size distribution showing the percent of particle mass smaller than the aerodynamic particle diameter. The respirable particle mass fraction is determined from the particle size distribution. The total particulate mass concentration also can be calculated since the impactor collects all particles.

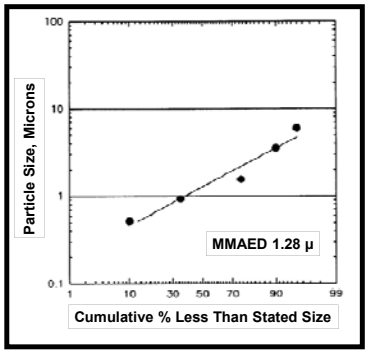
http://www.newstarenvironmental.com/pdfs/74126marpleSeries290_brochure.pdf 4-7

IMPACTOR PRINCIPLE OF OPERATION:

The flow enters the inlet cowl and accelerates through the six radial slots in the first impactor stage. The cowl eliminates ashes and debris from the sampler. Particles larger than the cut point of the first stage impact on the precut collection substrate. Then, the air-stream flows through the narrower slots in the second impactor stage, smaller particles impact on the second collection substrate, and so on. The widths of the radial slots are constant for each stage but are smaller for each succeeding stage. Thus, the jet velocity is higher for each succeeding stage, and smaller particles eventually acquire sufficient momentum to impact on one of the collection substrates. After the last impactor stage, remaining fine particles are collected by the built in 34 mm filter.



* http://www.unc.edu/courses/2007fall/envr/416/001/homework/smoketest.htm 4-8



http://www.aerosol.com.tw/product_277008.jpg

Particle size distribution, obtained using an Andersen impactor, as shown on the previous page. To obtain σ_g, divide the size at 84% (3.0 µm) by the size at 50% (1.28 µm) and you get 2.3.

4-9

CHAPTER 5: ACUTE INHALATION TOXICOLOGY

A. *LC50*

The LC50 is the atmospheric concentration, statistically estimated, to kill 50% of the animals when exposed for a fixed time period and observed for a specified post-exposure observation period.

EXPOSURE	POST-EXPOSURE (↓↓↓↓)
EXPOSURE (↓↓↓)	POST-EXPOSURE (↓↓↓)

Note: Deaths at arrows

5-1

Exposure: 1 hour, Observation period 14 days.

Exposure: 4 hours, Observation Period 14 days.

Exposure Time: Fixed, it can be 1, 4 or 8 hours or anything you want.

No death should occur during exposure so that all animals are exposed to the same "dose", but this is seldom achieved.

Observation period: Fixed, it can be anything, 14 days is not magic.

Exposure Concentration: Varies, from low to high, to arrive at lethality between 0 and 100% and then statistically calculate LC50.

5-2

Proper terminology is: LC50 for Chemical X is: 100 ppm for a 2-hour exposure period and 14 days post-exposure observation period. Both the exposure period and the post-exposure period must be given.

ALARIE'S RULE: The only appropriate method to calculate an LC50 (or LD50) and the 95% C.I. is as developed by Thompson and Weil. See: Weil, CS (1952), Biometrics 8: 249-261. Maximum of 4 animals/group and minimum of 4 groups of animals are needed. Thus 16 animals, or at most 6 groups for a total of 24 animals. Don't fool around getting an "approximate LC50" to save a few animals. Consult this reference and do it right. A more recent paper giving a computerized method for the above is: Schaper, MM, Thompson, RD and Weil, CS (1994). Computer programs for calculation of median effective dose (LD50 or ED50) using the method of moving average interpolation. Arch. Toxicol. 68: 332-337.

5-3

B. *LT50*

The LT50 is the time for 50% of the animals to die at a *particular exposure concentration*, also called "median time to death". Often used for saturated vapor concentration (Cs) of a liquid (i.e., maximum amount that can be present in air as described previously) to simulate a spill. Not a measure of toxicity (potency) because the answer is time, not amount. It is not a statistically calculated number with 95% C.I. as with the LC50.

5-4

The way to obtain it is simple. A single group of animals is used, always an odd number to simplify things. Most often 9 animals are used. The time at which the 5th death occurs is the LT50 (i.e., 4 died before the 5th and 4 will die after the 5th) thus the time at which the 5th died is the median time to death regardless of the time at which the last four die.

EXPOSURE (↓↓↓↓)	POST-EXPOSURE	5/9 Deaths
EXPOSURE (↓↓↓)	POST-EXPOSURE (↓↓)	5/9 Deaths

Note: Deaths at arrows

5-5

- **Time: Variable in the sense that the 5th death should occur during exposure, but sometimes this does not happen**
- **Concentration: Fixed, usually the maximum that can exist (i.e., Cs)**
- **Fast-acting, slow-acting, hazard concept at a particular exposure concentration**

Note: Resembles anesthetic gases, for comparisons of their speed of induction (time for a given level of anesthesia at a given concentration). Not their potency. Potency is how much in air (or blood) to produce a given level of anesthesia. Here we want to know, if a spill occurs, how fast people will die.

5-6

C. *LCT50*

This number is obtained by multiplying the LC50 by the duration of exposure. Thus the units will be ppm x min or mg/m^3 x min.

ALARIE'S RULE: The above is nonsense. By multiplying concentration by time you give the same weight to both variables and this is improper. Don't accept this. Insist on knowing both the exposure concentration and the duration of exposure. Furthermore, insist on getting the observation period, it may be different when comparing chemicals. Fortunately this has gone away, but you may encounter it in the older literatures or older reports.

5-7

D. *HABER'S RULE*

Simply stated, Haber was asking himself "if the LC50 for a chemical is 100 ppm when the exposure duration is 4 hours, would it be 200 ppm when the exposure duration is 2 hours or 400 ppm when the exposure duration is 1 hour?" Thus, the level of response would be: C x T = k. Certainly a good question to ask. It does work, within limits; don't change 100 ppm and 4 hours by more than a factor of three (i.e., 10 ppm for 40 hours will not work).

Note: The problem with this rule is that it works best for slow-acting substances, like phosgene, the war gas he was investigating. Most people quoting this rule never took the time to read the original article. Here it is: Haber, F (1924). "Funf Vortrage aus den Jahren 1920-1923:" No. 3, Die Chemie im Kriege; No. 5, Zur Geschichte des Gaskampfes, Julius Springer, Berlin. By the way, Haber won the Nobel Prize. Not for Haber's rule or for his work on chemical warfare. Do you know why he won the prize? His discovery is the most important for feeding the world today. No, it is not about pesticides and we still use his original discovery today in the exact same way.
Extra points on the exam if you know what it is!

5-8

E. *COMPARISON OF LC50 VALUES*

Often we are asked to compare LC50s of different chemicals and make a decision. Here are the rules:

1. Regardless of "statistical significance", the LC50s for two chemicals are comparable if within a factor of three.
2. If above a factor of three, start to think about it.
3. If above a factor of ten, it will definitely make a difference in real life situations if there is a spill.

F. *COMPARISON OF LT50 VALUES*

You really cannot compare these values for different chemicals. You use the LT50 to get an idea of rapidity of action at a particular exposure concentration, usually Cs.

5-9

G. *COMPARISON OF LC50 and LT50 VALUES SIMULTANEOUSLY*

Surprisingly, this can be done. However, it can be done only if you set appropriate rules first.

1. An exposure time period must be selected, relevant to the situation being investigated, and the post-exposure period *must be shorter than the exposure period* so that delayed deaths are not influencing the results. In the example provided here, 30 minutes was selected for the duration of exposure and 10 minutes for the post-exposure observation period.

5-10

2. Then the LC50 is determined, within a concentration range so that 50% of the animals will die close to the end of the exposure period selected. This time is taken as the LT50. This time will be the LT50, 22 minutes, in the example for wood smoke, when the amount of wood (64 grams, the LC50) needed to produce sufficient smoke to produce this effect is used.

3. Then all other materials are tested with the same protocol and can be compared to the initial material, in this case wood.

4. In the plot (see 5-14), the LC50 for wood (64 grams) and the LT50 for wood (22 minutes) are presented and the results for all other materials tested under similar conditions are presented. Very easy to compare.

5-11

5. Note that in making comparisons, the "As toxic as wood" uses a factor of three (divide 64 by 3 and multiply 64 by 3 and cheat a little and you get 20 and 200).

6. Note that in making comparisons, the "As fast acting as wood" uses a different factor. No good rule to use here, but use one that makes sense.

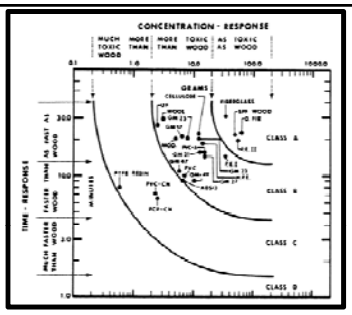
7. Finally, quadrants are drawn on this figure, beginning on the concentration axis and ending on the time axis and labeled Class A, B, C and D.

5-12

Now, in the Class A quadrant, we cannot say these materials are "as toxic as wood" or in the Class B quadrant, we cannot say they are "more toxic than wood". Because both "amount to kill" and "time to kill" influence where the data point is plotted for each material and by definition, "amount to kill" is toxicity. So, all we can say is that a material in Class A is "similar to wood". A material in Class B is less desirable than wood, etc.

Note: This concept of "exposure concentration to produce a given level of effect" and the "time required to produce it" is not unique to inhalation toxicology or aquatic toxicology but applicable to toxicology in general. Consider chronic carcinogenicity studies. What is the dose necessary to induce a particular tumor in 50% of the animals and what is the required duration to do so? In general, toxicology is dominated by amount. "Only the dose makes the poison." Much less attention is paid to "time required".

5-13



In this figure, each point represents the amount of material (grams on the X axis) which produced sufficient smoke to kill 50% on the animals (LC50) and the time (minutes on the Y axis) required to kill 50% of the animals (LT50) using that amount of material. To combine both LC50 and LT50 quadrants separate the materials in classes A, B, C and D. From Alarie, Y and Anderson, RC (1981). Toxicologic classifications of thermal decomposition products of synthetic and natural polymers. Toxicol. Appl. Pharmacol. 57: 181-188. Reprinted with permission from Academic Press.

5-14

H. *USE OF LC50 AND LT50 IN REGULATIONS*

As far as I know, the LT50 has never been used. The LC50 is used (among other data such as RD50) by the Occupational Safety and Health Administration (OSHA) and by the National Institute for Occupational Safety and Health (NIOS) to establish "Immediately Dangerous to Life or Health (IDLH)" exposure concentration to chemicals.

See: http://www.cdc.gov/niosh/idlh for documentation

5-15

Unfortunately there are three definitions for IDLH, depending upon the circumstances:

1. Hazardous waste and emergency response regulation (OSHA)
2. Permit-required confined spaces regulation (OSHA)
3. Respirator selection process (NIOSH). IDLH concentrations have been established by NIOSH for 387 chemicals. Do not believe that IDLH values published by NIOSH mean that death will occur at the IDHL. Make sure that you read the above web site before you use these IDLH values.

5-16

When using LC50 data, NIOSH prefers a 30-min exposure (certainly reasonable if we are talking about immediate death) and adjusts data from the literature to an equivalent 30-min value from other durations of exposure using the following equation:

$$\text{Adjusted LC50 (30-min)} = \text{LC50 (t)} \times (t/0.5)^{1/n}$$

Where: LC50 (t) = LC50 determined for t hours of exposure

n = a constant

The value for n selected by NIOSH is 3, based on a study by ten Berge[a]. This brings us back to Haber's Rule. Great progress!

[a]ten Berge, WF, Zwart, A and Appleman, LM (1986). Concentration-time mortality response relationship of irritant and systematically acting vapours and gases. J. Haz. Mat. 13: 301-309.

5-17

CHAPTER 6: EXPOSURE CHAMBERS

Principles to operate inhalation exposure chambers were presented by Silver in 1946. Also consult a few more recent but still old references. Read them before starting an inhalation study.

Silver, SD (1946). Constant gassing chambers: principles influencing design and operation. J. Lab. Clin. Med. 31: 1153-1161.

MacFarland reviewed Silver's article and expanded it.

See: MacFarland, HN (1983). Designs and operational characteristics of inhalation exposure equipment - A review. Fund. Appl. Toxicol. 3: 603-613.

There is also a report with many details.

See: Fraser, DA, Bales, RE, Lippman, M and Stokinger, HE (1959). Exposure chambers for research in animal inhalation. Public Health Monograph No. 57.

See also: Drew, RT and Laskin, S (1973). Environmental inhalation chambers. In: Methods of Animal Experimentation. W. I. Gay, Ed., Academic Press, N.Y., p. 1-41.

For more recent coverage: McClellan, RO and Henderson, RF (1995). Concepts in Inhalation Toxicology. Taylor and Francis.

6-1

A. *BEST DESIGN*

There is no such thing as a "best design" chamber to answer all the needs of inhalation toxicology studies. The "best design" is a chamber that works according to the following criteria:

- **Fairly uniform distribution of contaminants, variation of 10-15% between sampling ports, is quite acceptable. Forget the idea that you have to re-invent an exposure chamber because the concentration in the upper corners is higher than in the middle. There are no rats in the upper corners! Just sample above where the rats are.**

6-2

- **Airflow, going through the chamber (Liters/minute) being equal to the total volume (Liter) of chamber, is a good bet to run a chamber. Thus, a 100 L chamber should be operated at an airflow of 100 L/min. This is amply sufficient to prevent a temperature rise due to animal body heat and will also keep CO_2 and ammonia levels low. If this airflow presents a problem (e.g., high cost of pollutant, availability of pollutant, cleaning, etc.), it can be reduced, but probably not below 0.2 x the total volume of the chamber, without creating problems with temperature, ammonia, CO_2, humidity, etc.**

6-3

- **Animal load should be lower than 1% to 5% of the chamber volume. Calculate as follows: take the body weight of each animal to be same as its volume (i.e., 200 gram rat = 0.2 Liter) and multiply by number of animals. Thus, to expose 100 rats each weighing 200 grams (20 liters of rats), you need a minimum chamber volume of 2,000 liters. With non-reactive gases such as CO, etc., or vapors such as benzene, carbon tetrachloride, etc., this will work fine. Preferably, there will be only *one layer* of animals. This can be reduced to a 400 liter chamber (5%), but you are likely to need 2 layers. One layer is preferable if you are working with highly reactive gases which will react with the fur of animals or when you are working with large particles, but is not necessary with aerosols around 1 µm.**

6-4

- **Do not use the chamber for mixing. The added pollutant should be mixed with the incoming diluting air prior to entering the chamber or mixed at the top or entrance of the chamber. The idea of placing baffles, etc. in a chamber for better mixing is nonsense. This simply provides more surface area for interaction with gases or aerosols. A baffle can be used to prevent large particles from entering the chamber or at the entrance of the chamber for proper mixing, but *never* within a chamber.**
- **Do not use a fan in a chamber for mixing. A fan will increase turbulence which will cause aerosol coagulation, stratification and deposition on various surfaces in the chamber.**

6-5

- **With reactive gases, "conditioning" of the chamber should be done prior to loading animals in the chamber. Once this is done, addition of animals may drop the concentration by at least 50% and sometimes by as much as 95%. This is not serious for a long-term chronic study. Indeed during the first week of such a study, adjustment can be made to the delivery system to bring the concentration upward to the desired level. In cases of acute studies, if you want to know what the animals are likely to "soak", collect dead animals used for other experiments, keep them refrigerated and then place them in your chamber. There will be no difference between live and dead animals since the amount of pollutant inhaled by the living animals is extremely small; the major contribution comes from reaction with the fur. The best design is nose or head-only exposure for these situations, reducing the possibility of interference.**

6-6

- **Permitted daily variation in exposure concentration for chronic studies :**

Reactive gases	**± 20% of desired**
Non-reactive gases	**± 15% of desired**
Aerosols < 1 µm	**± 20% of desired**
Aerosols 1-5 µm	**± 20% of desired**

"Big Problem"

Aerosols >5 or 10 µm: Don't use them in large chambers; nose or head-only exposures are more appropriate. However, relevance is in question, rats do not inhale rocks!

6-7

NOTE: Often, consumer spray cans products or spray paints need to be tested. Such products are often called "aerosol cans". These do not produce aerosols. The particles coming out of them are too large to the considered aerosols. They are "spray cans".

Alarie's Rule is: Don't spray paint rats and think this is an "inhalation exposure". Nonsense.

More details below about appropriate particle size to use. Remember, an inhalation chamber is not a spray painting booth. The only solution to this problem is to set up elutriation systems to remove the large particles or to reformulate the aerosol delivery system so that the particle size is appropriate for an *inhalation* study. Obviously this will not be exactly the same as what consumers will be exposed to. However, without doing this you may end up with an LC50 of 1 ton/m^3. While this may be reassuring, it is not appropriate, since none of the particles may have been inspirable or respirable for the exposed animals. Why do this? EPA is now requesting that appropriate aerosol size be used and it is about time. If you are reviewing an old study and no information is given on particle size, you should be very careful.

6-8

B. DESIGN FOR VERY HIGH LEVEL OF AEROSOL EXPOSURE

The decision to use a chamber or head- (or nose-) only exposure is impossible to make without some preliminary work with the chemical. This work can be done using a small chamber and a few animals. In general, concentrations higher than 500 mg/m^3 are difficult to work with, unless the particle size is small. Head-only or nose-only exposures are indicated. This presents some problems; cage control and head-only or nose-only exposure control must be used. This results in high labor costs, but it can be done well and has been done well.

6-9

There is an EPA recommended level of 5 gram/m^3 of aerosol as a level, at which if no deaths occur, the chemical would be considered "non toxic". This is impossible to achieve for most solids or liquids <u>and</u> keeping the particle size reasonably small for animals to inhale <u>and</u> at the same time, using an inhalation chamber with the particle size and exposure concentration remaining fairly uniform from top to bottom of the chamber. Don't waste your time trying this. A very bad idea. Just as bad as toxicologists injecting animals with the "maximum tolerated dose" and then chasing artifacts created by such nonsense.

6-10

The following guidelines are from personal experience.

<50 mg/m^3	**smaller particles (1-3 µm): OK** **larger particles (up to 10 µm): OK**
>50 mg/m^3	**smaller particles: OK** **larger particles: more difficult**
500 mg/m^3	**smaller particles: OK** **larger particles: they will deposit**
5,000 mg/m^3	**smaller particles: OK** **larger particles: too much deposition**

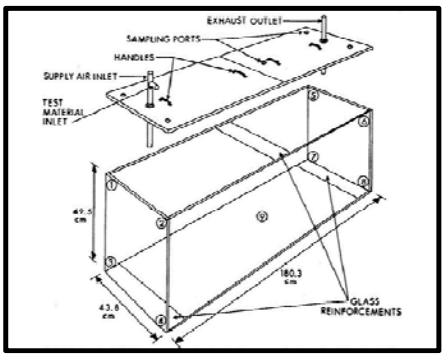
Note: Consult the following article for problems related to achievable aerosol exposure concentrations with realistic particle size: Recommendations for the conduct of acute inhalation limit test. Fund. Appl. Toxicol. 18, 321-327, 1992.

6-11

C. *CHEAP CHAMBER, GOOD RESULTS*

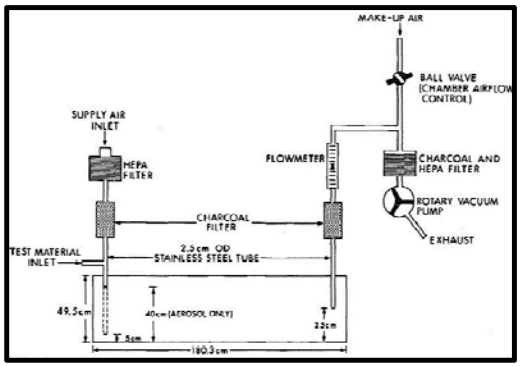
For acute, sub-acute or chronic work using 10-20 rats or 20-50 mice, an all-glass 100-liter aquarium is just perfect. A cover can be made out of plywood or Plexiglas with the inside of the cover lined with Teflon or polyethylene film. Such a chamber costs about $100 with all the fittings. Operated at 100 Liters/minute, an equilibrium concentration will be reached in about 5 minutes (see below) with uniform distribution of contaminants, gases or aerosols. It is not necessary to use expensive stainless steel chambers with pyramidal tops and bottoms as shown below. Simple, and it works!

6-12



See: Barrow, CS and Steinhagen, WH (1982). Design, construction and operation of a simple inhalation exposure system. Fund. Appl. Toxicol. 2: 33-37.

6-13



See: Barrow, CS and Steinhagen, WH (1982). Design, construction and operation of a simple inhalation exposure system. Fund. Appl. Toxicol. 2: 33-37.

6-14

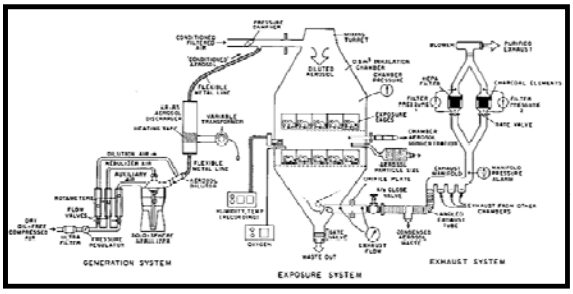
D. *TYPICAL INHALATION CHAMBER*

The figure shown on the next slide illustrates a typical inhalation chamber that can be used to expose a large number of animals.

You will note that it has a pyramidal top and bottom (also called a "Rochester chamber", designed at the University of Rochester).

Such chambers are usually built with stainless steel and a glass door to observe the exposed animals.

6-15



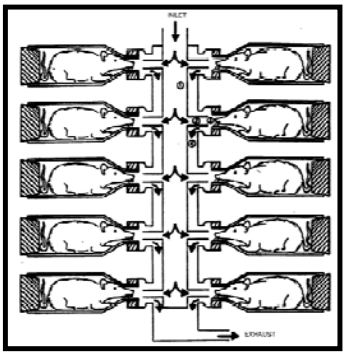
From: Miller, JL, Stuart, BO, Deford, HS, and Moss, OR (1981). Liquid aerosol generation for inhalation toxicology studies. In: Proceedings of the Inhalation Toxicology and Technology Symposium. B.K.J. Leong, Ed., Ann Arbor Science Publishers, Ann Arbor, MI, pp. 121-138. Reprinted with permission.

6-16

E. *NOSE-ONLY EXPOSURE CHAMBER*

The figure on the next slide shows a typical "nose-only" exposure chamber. It prevents contamination and/or reaction with the fur of the animals. It also reduces the volume of the "exposure chamber" which is an advantage when working with small quantities of available chemicals, expensive chemicals or radioactive chemicals. With a reduced volume chamber, equilibrium time (see below) is achieved quickly and thus this chamber is very suitable for short exposure experiments.

6-17



From Cannon, WC, Blanton, EF and McDonald, KE (1983). The flow-past chamber: an improved nose-only exposure system for rodents. Am. Ind. Hyg. Assoc. J. 44: 923-928. Reprinted with permission.

6-18

F. *DO NOT EXPOSE ANIMALS, UNLESS YOU ARE SURE*

Prior to exposing animals, you should have good experience with an empty chamber, including the housing cages and the contaminant delivery system, as well as the method for analysis of the contaminant. If you are dealing with an aerosol, determination of the particle size needs to be done. You should know how large the difference is between the "nominal concentration" (as calculated from contaminant flow entering the chamber and the chamber airflow) and "actual concentration", as obtained from appropriate sampling of air within the chamber and appropriate chemical analysis.

6-19

G. *RAPID GUIDE FOR EQUILIBRATION TIME AND ITS USE*

As a gas or an aerosol is introduced at a uniform rate in an exposure chamber maintained at a continuous airflow, the concentration within the chamber increases until it is practically constant (equilibrium concentration, t_{99}). Assuming perfect mixing, from Silver (see reference above):

$C = (w/b)\ (1\text{-}e^{-bt}/a)$
C = Concentration in mg/liter , at time t
w = Milligrams of contaminant introduced in the chamber/minute
a = Volume of the chamber, in liters
b = Volume of air through the chamber, Liters/minute
e = The base of the natural log, 2.7182
t = Time, in minutes

Using this equation: t_{99} *(minutes) = 4.6 x (a/b)*

6-20

Thus, when *a* and *b* are equal, Period "X" in the figure below is about 5 minutes. In practice, we want Period "X" to be smaller or equal to 10% of Period "Y". Note that when the exposure is terminated, we also have a time period (Period "Z") during which the animals are still exposed.

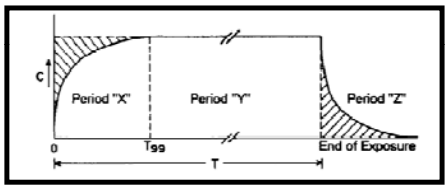


Illustration of time to reach equilibrium (or t_{99}) in an exposure chamber. From RT Drew. The design and operation of systems for inhalation exposure of animals. In: Toxicology of Inhaled Materials. Witschi, HP and Brain, JD, Eds. Springer-Verlag, NY, 1985, 1-22. Reprinted with permission.

6-21

H. *AIR CHANGES*

An air change is said to occur when a volume of air, equal to the volume of the chamber, has passed through the chamber (i.e., *a* = *b*). This is a convenient term used by ventilation engineers but is not correct. When such occurs, from the above equation, only 63% of the air has been "changed". So forget about this. Give us *a* and *b*.

Note: With all the concerns about indoor air quality, you will encounter "air change" as used by ventilation engineers. A typical old house may have one air change/hour. A mobile home may have about 0.02-0.5 air change/hour, very little air infiltration. A modern office with air conditioning will have 5-8 air changes/hour and a modern animal room will have 8-12 air changes/hour.

6-22

CHAPTER 7: EXPOSURE SYSTEMS FOR CALCULATION OF DOSE

In order to calculate the net dose received during exposure to aerosols or reactive gases, the exposure concentration and duration of exposure are needed. Both are available using the exposure chambers described above. However, two additional variables are needed: tidal volume (Vt, amount of air inspired with each breath) and the breathing frequency (breaths/minute, f, or BPM). These cannot be obtained with the exposure systems described above.

7-1

Vt and f can be obtained with head-only exposure systems, with the body of the animal held in an enclosure having a seal at the neck of the animal. Such a device is called a "head-out body plethysmograph" or simply, a body plethysmograph.

Note: A plethysmograph is an instrument for measuring and recording the variation in volume of a part of the body; it consists of a closed vessel surrounding the part of the body to be measured. Connected to the vessel is an instrument for measuring volume increases and decreases within it. For a body plethysmograph, thoracic volume displacement is measured and it is assumed that the amount of air entering the lung with each breath is the same as thoracic displacement, thus representing Vt.

7-2

Note: The thoracic volume displacement can be measured in two ways. A pressure transducer can be attached directly to a port on the plethysmograph; the change in pressure created with each breath from thoracic displacement will be proportional to the change in volume. A pneumotachograph, to which a differential pressure transducer is attached, is connected to a port on the plethysmograph.

A pneumotachograph is a device for measuring the rate at which air flows in and out of the plethysmograph due to thoracic displacement with each breath. The rate of airflow (mL/second) when integrated with time yields volume (ml) and a record of tidal volume is obtained.

There is also another way to obtain Vt and f. Here, the entire animal is within a sealed exposure. This is then called a "whole-body plethysmograph". The advantage is that there is no restraint at the neck, but a little trickier to use.

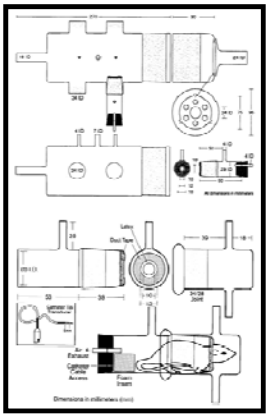
These systems are described below and further details are given in: Alarie, Y Iwasaki, I and Schaper, M (1990). Whole body plethysmography in sedentary or exercise conditions to determine pulmonary toxicity, including hypersensitivity induced by airborne chemicals. J. Am. Coll. Toxicol. 9: 407-439.

7-3

Top: Glass exposure chambers, showing attached glass body plethysmograph for exposure.

Bottom: Glass body plethysmograph, with head dome attached for exposure. A pressure transducer or pneumotachograph is attached to the port of the plethysmograph to measure tidal volume. As also shown at the bottom, a catheter tip transducer can be inserted in the thoracic cavity of the animal to measure transpulmonary pressure.

This additional measurement permits the evaluation of pulmonary flow resistance (RL) and dynamic lung compliance (DynL) when needed.

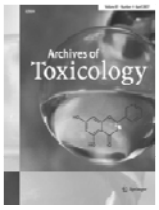


7-4

Note:
Figures (top, bottom) on page 7-4 taken from:

* Vijayaraghavan, R, Schaper, M, Thompson, R, Stock, MF, and Alarie, Y (1993). Characteristic modifications of the breathing pattern of mice to evaluate the effects of chemicals on the respiratory tract. Arch. Toxicol. 67: 478-490.

** Vijayaraghavan, R, Schaper, M, Thompson, R, Stock, MF, Boylstein, LA, Luo, JE and Alarie, Y (1994). Computer assisted recognition and quantitation of the effects of airborne chemicals at different areas of the respiratory tract in mice. Arch. Toxicol. 68: 490-499.



http://www.springer.com/biomed/pharmacology+%26+toxicology/journal/204
*http://link.springer.com/article/10.1007/BF01969919
**http://link.springer.com/article/10.1007/s002040050101

7-5

CHAPTER 8: FACTORS INFLUENCING THE DOSE FOR AEROSOLS

A. *FACTORS INFLUENCING THE TOTAL DOSE*

1. **Concentration in air, *C* (mg/m³)**
2. **Minute ventilation, *VT x f* (mL/minute)**
3. **Duration of exposure, *t* (minutes)**
4. **Deposition fraction, *α* (%)**
   Deposition: all factors which determine what fraction of the inspired aerosol will be caught in the respiratory tract (nose to alveoli) and fail to exit with the expired air. Deposition is always given as the probability that an inhaled particle *of a given size* will fail to be exhaled.

$$Retained\ Dose = \alpha \times VT \times f \times C \times t = mg$$

8-1

Table 5. Useful Values at Rest for Vt and f [a]

Species	Vt (mL)	f (breaths/minute)
Man (70 kg)	800	15
Mouse (.02 kg)	0.1-0.2	250
Rat (0.3 kg)	2	120
Guinea Pigs (0.4 kg)	2	90
Monkeys (3 kg)	40	35
Man (Moderate Exercise)	1,450	18

[a] Useful values for various animal species for a large number of variables concerning the respiratory system can be found in: Parent, R A. (1992). Comparative biology of the normal lung. CRC Press, Boca Raton, FL., 830 pp.

For lung surface area in humans, use 70 m^2.
A value to remember is the total amount of air breathed/day by a normal adult (70 kg body weight): 20 m^3 used by the EPA to calculate the "reference dose".

8-2

Required reading for anyone planning to work with aerosols:

Task Group on Lung Dynamics (1966). Deposition and retention models for internal dosimetry of the human respiratory tract. Health Physics 12: 173-207.

A good review is:
Brain, JD and Valberg, PA (1979). Deposition of aerosol in the respiratory tract. Amer. Rev. Resp. Disease 120: 1325-1373.

Excellent books on aerosols are:
Willeke, K, and Baron, PA (1993). Aerosol Measurement. Principles, Techniques and Applications. Van Nostrand Reinhold, NY.

Hinds, WC (1999). Aerosol Technology. Properties, Behavior and Measurement of Airborne Particles. John Wiley, NY.

8-3

Using the above formula and values for *VT and f,* you can assume 50% (0.5) for *α* if the particle size is not known in order to get a "ball park" figure. Thus, if a human is exposed at a concentration of 1 mg/L for a period of 60 minutes and has a *Vt* of 800 mL and *f* of 15/minute, the total dose received would be:

0.5 x 800 mL x 15/minute x 1 mg/1000 mL x 60 minutes

= 360 mg

8-4

This same formula can also be used for fibers. The 2014 TLV®-TWA for asbestos fibers (length > 5 µm; aspect ratio > 3:1) is 0.1 fiber/cc. If a human is exposed for 8 hours at this concentration, the total number of fibers deposited would be: 288,000 fibers.

Note: This gives only total deposited. This does not give the regional deposition which is highly important. Also, this does not take clearance into account, which takes place simultaneously during the exposure period. (See below).

Note: When the above simple formula is used, make sure the units are properly matched. A common mistake is to forget that exposure concentration is usually given in mg/m^3 and Vt is given in liter or mL. There are 1,000 liters in each m^3 and 1,000 mL in each liter! Expert scientists have used the above formula and published results forgetting this.

8-5

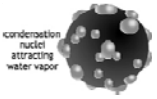
B. *FACTORS AND MECHANISMS AFFECTING TOTAL DEPOSITION AND THE REGIONAL DEPOSITION OF AEROSOLS*

Key factors affecting deposition sites in the respiratory tract are:

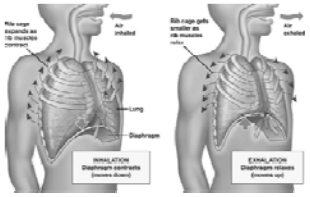
- **Size, Shape and Density (of the particle) The above 3 characteristics are taken into account simultaneously for particles > 0.5 µm by experimental determination of their aerodynamic mass equivalent diameter and for particles <0.5 µm only the size is important, as already presented.**

8-6

- **Hygroscopicity (depends on chemical composition) since a small particle can grow upon entering the 100% RH of the respiratory tract.**



- **Pattern of pulmonary ventilation including nose vs. mouth breathing, as well as volume (Vt) and frequency (f) of each breath**

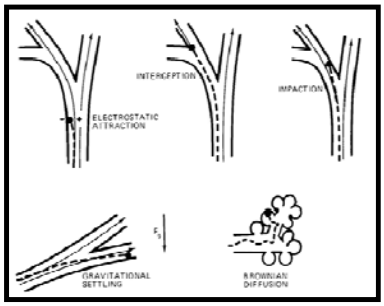


8-7

There are 5 major physical mechanisms by which airborne particle deposition occurs in the respiratory tract.

These are depicted in the diagram in the next slide and are explained below. For impaction, sedimentation and diffusion, mathematical equations have been developed long ago to estimate how particles will deposit in the respiratory tract and are used to estimate the location of deposition in the respiratory tract according to particle sizes.

8-8



This figure illustrates the five major mechanisms involved in particle deposition along the respiratory tract.

From Raabe, OG (1980). Pulmonary toxicology of respirable particles. DOE Symposium. Series 53, Technical Information Center, U.S. Dept. of Energy, pp 1-28. Reprinted with permission.

8-9

1. Impaction, Inertia
When an obstacle exists in the path of the airflow, or bifurcations or tortuous paths occur such as in the nose or tracheobronchial tree, small particles will follow the air flow lines, but large particles, because of greater inertia are unable to change direction and will impact. Most important mechanism of deposition for particles > 3-5 µm in the nasopharyngeal area and central airways of the lung.

2. Sedimentation, Settling
All particles with density greater than air experience a downward force, due to gravity. Important along the conducting airways of the lung and alveolar level.

3. Diffusion, Brownian Movement
Motion caused by random molecular collision. Important for particles < 0.5 µm and most important at the alveolar level.

8-10

4. Electrostatic Attraction
All aerosol particles have a positive (+) (non-metallic) or a negative (-) (metallic) charge which may affect their deposition. However, little is known about the effect of charge, except for highly charged particles which can occur in freshly generated particles. Important for polymeric fibers, proteins (synthetic or natural) which can hold a high charge upon friction. Depending on chemical composition, also important for nanoparticles.

5. Interception
Of importance for fibers as the inspired air comes in close contact with a surface. Fiber deposition models are less well developed than for particles.

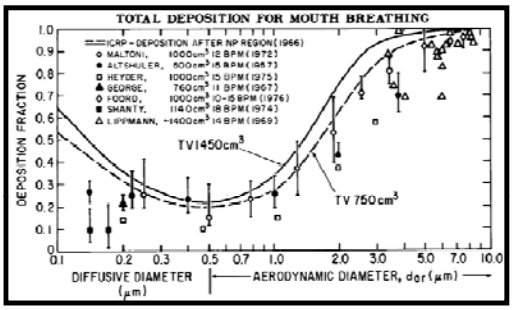
8-11

C. *DEPOSITION CALCULATIONS AND RESULTS*
From the above, calculations of deposition according to aerodynamic particle size have been made for the various regions of the respiratory tract:

1. **Nasal-Pharyngeal (N-P)**
2. **Tracheo-Bronchial (T-B)**
3. **Pulmonary (P)**

These are presented in the figures that follow and a quick summary is also presented in a table in the next slide.

8-12



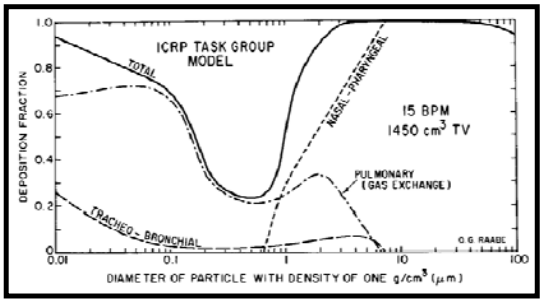
From Raabe, OG (1984). Size-selective sampling criteria for thoracic and respirable mass fractions. Ann. Am. Conf. Ind. Hyg. 11: 53-65. Reprinted with permission.

8-13

This figure shown on the previous slide, 8-13, presents the results of calculations of total deposition past the nasopharyngeal region using mathematical equations to estimate deposition (solid curves) in humans and experimental results from various investigators. You can see that there is a good agreement above 0.25 µm. Note that the author uses the terminology "diffusive diameter" and "aerodynamic diameter" for particles < or > than 0.5 µm. Quite correct, but rarely used.

Unlike "total deposition" presented in the figure on 8-13, we have no experimental data for "regional" deposition in humans. The figure on the next slide, 8-15, presents the calculated deposition in the three areas of the respiratory tract. You can also see here, as well as in the previous figure, that if you use 50% for % deposition the error cannot be more than a factor of 2.

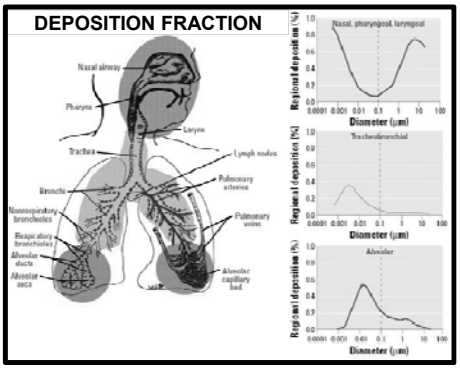
8-14



This figure gives you the calculated deposition in each region of the respiratory tract, according to particle size, and adding each region gives you total deposition.

From Raabe, OG (1984). Size-selective sampling criteria for thoracic and respirable mass fractions. Ann. Am. Conf. Ind. Hyg. 11: 53-65. Reprinted with permission.

8-15



A more recent publication (Oberdörster, 2005), also illustrating particle deposition patterns as shown in 8-15.

8-16

Quick Summary of Deposition[a]

Areas	Directional changes	Air Velocity	Cross-Sectional Area	Residence Time
Naso-pharyngeal	Abrupt	++++	cm^2 +	Brief
Trachea	Nearly straight	+++	cm^2 +	Brief
Bronchial	Abrupt	++	10 cm^2 +	Brief
Alveolar	Mild	+	10^5 cm^2	Large

[a]From Cassarett, Modified by McClellan and Re-modified

8-17

Quick Summary of Deposition[a]

Areas	Importance of Mechanisms			Primary Particle Deposition
	Impaction	Sedimentation	Diffusion	
Naso-pharyngeal	+++	+	+	5-30 μm
Trachea	+	+	+	1-5 μm
Bronchial	+++	++	+	1-5 μm
Alveolar	+	+++	++++	<1 μm

[a]From Cassarett, Modified by McClellan and Re-modified

8-18

C. *MAN VS. LABORATORY ANIMAL*

With aerosol exposures and laboratory animals, it is important to recognize that although the same particle size may be used, deposition sites and clearance rates are likely to be different. Some general conclusions:

1. **The major factor controlling net dose received seems to be minute ventilation and, therefore, the small animals will receive a higher total dose because of their higher minute ventilation to body weight ratios.**

8-19

2. **The smaller the animal, the larger the probability of nasal retention. A 2 μm particle has about the same probability of being retained in the mouse nose as an 8 μm particle in man. Important for large particles.**

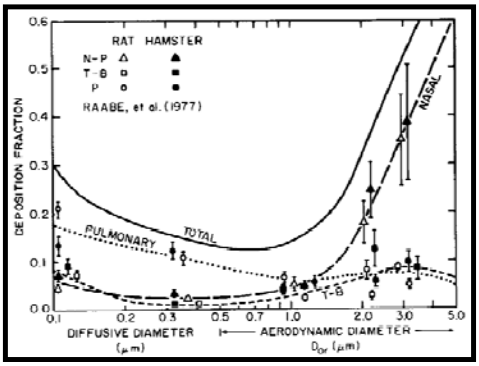
3. **The larger the particles are above 3 μm the more chance of difference in regional deposition with humans. Total net dose may be the same but biological effects may be different because the deposition sites and clearance rates are different. In particular, clearance rates vary widely between different species. In general, clearance in rats and mice is faster than in humans, monkeys or guinea pigs.**

8-20

4. **To have the same relation with humans between atmospheric particulate concentration and rate of deposition of particles in the *lungs*, fairly uniform particles around 1-3 μm (MMD) seem to be highest to use with small rodents, this would be defined as "inhalable" or "inspirable" and would also be classified as "respirable" particles for small laboratory animals. See below for the descriptions and definitions of these terms.**

8-21

Compare the results obtained below in rats and hamsters to the results obtained in humans previously presented in 8-15



8-22

The figure on 8-22 shows the deposition of monodispersed aerosol of fused aluminosilicate spheres in small rodents in the different areas of the respiratory tract, from analysis of each region after sacrificing the animal following exposure.

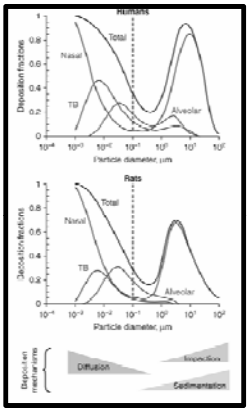
Figure on 8-22, from: Raabe, OG, Yeh, HC, Newton, GJ, Phalen, RF and Velasquez, DJ (1977). Deposition of inhaled monodispersed aerosols in small rodents. In: Inhaled Particles IV. Walton, WH (Ed.), Pergamon Press, NY. p.3.

Note: For reviews on comparison of deposition and clearance of inhaled particles in different animal species, consult:

Schlesinger, RB (1989).Deposition and clearance of inhaled particles. In: Concepts in Inhalation Toxicology. Hemisphere Publishing NY, pp.163-192.

Snipes, MB (1989). Long-term retention and clearance of particles in mammalian species. CRC Crit. Rev. Toxicol. 20: 175-211.

8-23



Model predictions of deposition fractions for different particle sizes in **humans** (top) and **rats** (bottom).

Nanosized particles are left of the dashed line (<100 nm). Their deposition is governed by diffusion (Brownian motion of air molecules).

This figure was provided by B. Asgharian.

From: Casarett and Doull's Toxicology (8th Edition, 2013), p.1206 (Figure 28-12)

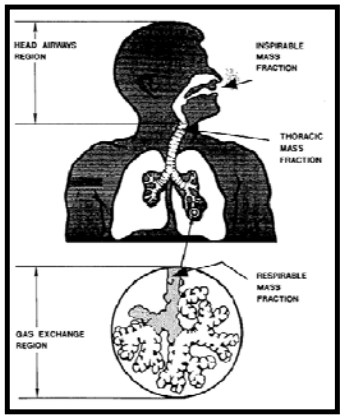
8-24

CHAPTER 9: NEW NOMENCLATURE

Name	Description
Inspirable Mass Fraction or Inhalable Mass Fraction	**Can enter into the head airways region when breathing via the nose or mouth. Older terminology: Nasopharyngeal deposition.**
Thoracic Mass Fraction	**Can penetrate past larynx. Older terminology: Tracheo-bronchial deposition.**
Respirable Mass Fraction	**Can penetrate past terminal bronchiole. Older terminology: Pulmonary deposition.**

Modified from Phalen, RF (1984). Introduction and Recommendations. Ann. Am. Conf. Ind. Hyg. 11: 23-26.

9-1



9-2

[a] Formal Definition of Each Fraction: IPM

Name	Definition
Inspirable Particulate Mass or Inhalable Particulate Mass (IPM)	**Consists of those particles that are captured according to the following collection efficiency regardless of sampler orientation with respect to wind direction:** $E = 50(1 + \exp\{-0.06\ d_a\}) \pm 10$ **for** $0 < d_a < 100$ **μm** **Collection characteristics for** $d_a > 100$ **μm are presently unknown. E is collection efficiency in percent and** d_a **is aerodynamic diameter in μm.**

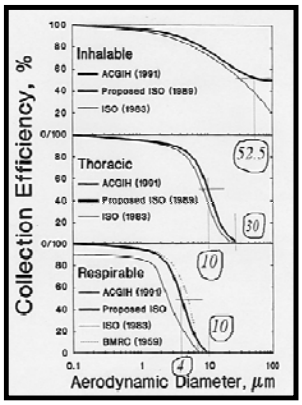
[a] Modified from American Conference of Governmental Industrial Hygienists: Particle Size - Selective Sampling in the Workplace. Pub. No. 0830, ACGIH, Cincinnati, OH, 1984. Re-modified 1996.

9-3

[a] Formal Definition of Each Fraction: TPM, RPM

Name	Definition
Thoracic Particulate Mass (TPM)	Consists of those particles that penetrate a separator whose size collection efficiency is described by a cumulative lognormal function with a median aerodynamic diameter of 10 μm ± 1.0 μm and with a geometric standard deviation of 1.5 (± 0.1).
Respirable Particulate Mass (RPM)	Consists of those particles that penetrate a separator whose size collection efficiency is described by a cumulative lognormal function with a median aerodynamic diameter of 4.0 μm ± 0.3 μm and with a geometric standard deviation of 1.5 (± 0.1).

9-4



This figure was taken from Willeke, K and Baron, PA. The figure differs from that of the ACGIH in that the word, "Inhalable" is used instead of "Inspirable", and 0.1 is used instead of 0 at the start of the X axis. This figure was modified to show the median cut-point (50%) for each fraction and the upper limit for the Thoracic and Respirable fractions. No upper limit is available for the Inhalable fraction, but is probably around 200 μm.

9-5

CHAPTER 10: CHARACTERISTICS OF AEROSOLS INFLUENCING THEIR TOXICITY

A. *MASS CONCENTRATION*
mg/m^3

B. *PARTICLE SIZE AND DISTRIBUTION*
MMD or MMAD

C. *SURFACE AREA AND LENGTH*
While mass deposited is a primary factor, other factors such as surface area, particle charge, fiber length, etc. may be important. Short asbestos fibers are much less potent than long asbestos fibers.

10-1

D. *SOLUBILTY - TRANSLOCATION*
How long the particles are retained in the respiratory tract can obviously have an effect on the degree and/or type of toxic effect.

1. Solubility: lipid or water soluble, non-reactive chemicals

If a particle is soluble in lipid or water, it will be absorbed rapidly into blood, more so if lipid soluble than water soluble. If absorbed from the pulmonary compartment, it is equivalent to an intra-arterial injection.

10-2

Chemicals having a lower molecular weight (MW) are absorbed into blood faster than those having a higher MW. Particularly important for drugs, and quite important for drug delivery for an effect on the lung tissue itself vs. using inhalation for systemic effect. For example, insulin is absorbed readily into blood when inhaled.

2. Translocation
This is a more general term and is more appropriate than solubility. It includes absorption into blood via solubility as given above, but also includes a variety of other mechanisms by which particles are cleared from the respiratory tract or absorbed into blood.

10-3

These include:

a) Reaction/s with biological molecules which may increase solvation or transport (carriers, etc.), but may also decrease transport
b) Ciliary movement (nasopharynx, trachea, bronchial tree, down to the terminal bronchioles) will move particles deposited on the mucus layer
c) Phagocytosis of particles by macrophages and clearance via ciliary- mucus transport
d) Transport of insoluble particles across alveolar epithelium into lymphatics

10-4

3. Categories of "solubility" or "translocation"

Three categories have been established:

Category Y	**Very Insoluble, Clearance in years Avidly retained**
Category W	**Somewhat Soluble Clearance in weeks Moderately retained**
Category D	**Very Soluble Clearance in minutes/days Minimally retained**

10-5

CHAPTER 11: RETENTION AND CLEARANCE

A. *MODEL*

A model proposed by the Task Group on Lung Dynamics in 1966* is used. This model is shown in the figure on 11-4 and includes the following items previously presented.

1. **Mass concentration in the air**
2. **Particle size to determine percentage deposition in each portion of the respiratory tract**
3. **Class of particles in terms of clearance time**

* Note: See 11-2 for references

11-1

B. *INPUTS FOR THE MODEL*

These are:

D_1 Total dust concentration in inhaled air (mg/m^3)

D_2 Total dust concentration in exhaled air (mg/m^3)

D_3 Total dust deposited in N-P compartment (mg)

D_4 Total dust deposited in T-B compartment (mg)

D_5 Total dust deposited in P compartment (mg)

* Task Group on Lung Dynamics (1966). Deposition and retention models for internal dosimetry of the human respiratory tract. Health Physics 12: 173-207. This model has been updated, adding great detail, but retaining the basic original principles.

ICRP Publication 66 (1994). Human Respiratory Tract Model for Radiological Protection. Pergamon Press. Also a very good source for respiratory tract anatomy, morphology and physiology.

11-2

C. *OUTPUTS FOR THE MODEL: CLEARANCE, HALF-LIVES, PATHWAYS AND MECHANISMS*

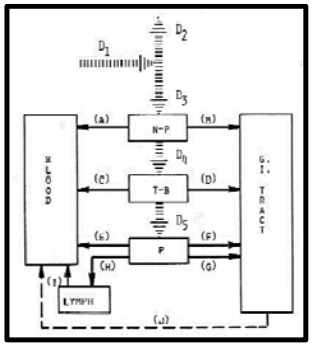
These are given in a table below in an attempt to summarize all of these items. First, look at the figure on 11-4. For each letter on this figure, there is an explanation for it in the table that follows on 11-5 through 11-8.

Use the half-lives for relative comparisons rather than absolute numbers.

11-3

This figure presents the input-clearance model. The large dashed arrows are particles, with inputs D_1, D_2, etc., as given on 11-2. Solid arrows with small letters represent the clearance pathways/ mechanisms as defined in the table on 11-5.

Note: The letters (a), (c), and (e) = soluble particle paths, while the letters (b), (d), (f), (g), (h), and (i) = insoluble particle paths. The letter (j) represents a path from the GI tract into blood.



11-4

Summary Table of Clearance, Half-Lives and Mechanisms[a]

Applicable Regions	Clearance Pathways	Mechanisms of Clearance and Sites Cleared To	Class of Particle[b]	Fraction of the Total Deposited Cleared by the Given Pathway	Biological Half-Time (Clearance) (minutes or days)
N-P	a	Uptake into systemic blood via solution	D W Y	0.50 0.10 0.01	4 min 4 min 4 min
	b	Uptake into GI tract via muco-ciliary transport	D W Y	0.50 0.90 0.99	4 min 4 min 4 min
T-B	c	Uptake into systemic blood via solution	D W Y	0.50 0.10 0.01	10 min 10 min 10 min
	d	Uptake into GI tract via muco-ciliary transport	D W Y	0.50 0.90 0.99	10 min 10 min 10 min

11-5

Summary Table of Clearance, Half-Lives and Mechanisms[a]

Applicable Regions	Clearance Pathways	Mechanisms of Clearance and Sites Cleared To	Class of Particle[b]	Fraction of the Total Deposited Cleared by the Given Pathway	Biological Half-Time (Clearance) (minutes or days)
P	e	Uptake into arterial blood via solution	D W Y	0.80 0.15 0.05	30 min 90 days 360 days
	f	Endocytosis by recruitable alveolar macrophages & uptake to GI tract via muco-ciliary transport	D W Y	NA 0.40 0.40	NA 1 day 1 day

11-6

Summary Table of Clearance, Half-Lives and Mechanisms[a]

Applicable Regions	Clearance Pathways	Mechanisms of Clearance and Sites Cleared To	Class of Particle[b]	Fraction of the Total Deposited Cleared by the Given Pathway	Biological Half-Time (Clearance) (minutes or days)
P	g	2nd pulmonary clearance process, much slower than f, but by same mechanisms	D W Y	NA 0.40 0.40	NA 90 days 360 days
	h	Slow movement of insoluble particles across alveolar epithelium to the lymphatics	D W Y	0.20 0.05 0.15	30 min 90 days 360 days

11-7

Summary Table of Clearance, Half-Lives and Mechanisms[a]

Applicable Regions	Clearance Pathways	Mechanisms of Clearance and Sites Cleared To	Class of Particle[b]	Fraction of the Total Deposited Cleared by the Given Pathway	Biological Half-Time (Clearance) (minutes or days)
Lymph/ Lymph Nodes	i	From lymph and lymph nodes into systemic blood. Lymph nodes are essentially "filters" for Class Y. Very important if particle is radioactive	D W Y	1.0 1.0 0.10	30 min 90 days 360 days

11-8

Summary Table of Clearance, Half-Lives and Mechanisms[a]

Notes

[a] **Prepared from Table 4 and the text of the IRPC Task Group on Lung Dynamics 1966 Report.**

[b] **Abbreviations used in table:**
D = Soluble
W = Somewhat Soluble, and
Y = Insoluble

[c] **The way to read the table is: for the N-P region, 50% of Class D particles deposited there will be cleared with a $T_{1/2}$ of 4 min into systemic blood while 50% will be cleared with a $T_{1/2}$ of 4 min into the GI tract, etc.**

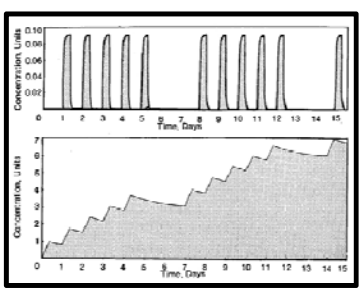
[d] **What is important here is not the numbers. Instead, look at this table to get a general appreciation of the differences between different types of particles according to their solubility.**

A similar table was prepared by Morrow, P E (1984). Pulmonary clearance. In: Advances in Modern Environmental Toxicology. Volume VIII. Occupational and Industrial Hygiene: Concepts and Methods. N.A. Esmen and M. A. Mehlman, Eds. Princeton Scientific Publishers, pp. 183-202.

11-9

D. *APPLICATION OF THE MODEL*

With the availability of computers, it is now easier to estimate aerosol clearance and accumulation in the respiratory tract.



Pulmonary concentration of particles as a function of time during occupational exposure. Top, Class D: Minimally retained Bottom, Class Y: Avidly retained

This figure shows a computer simulation of pulmonary retention depending upon the class of inhaled particles. From Brain, J D and Valberg, P A (1974). Models of Lung Retention Based on ICRP Task Group Report. Arch. Environ. Hlth. 28: 1-11. Reprinted with permission, Heldref Publications

11-10

The clearance of insoluble particles is greatly reduced at high exposure concentrations in chronic studies.

See: Morrow, P E, Haseman, J K, Hobbs, C H, Driscoll, K E, Vu, V and Oberdorster, G (1996). The maximum tolerated dose for inhalation bioassays: toxicity vs. overload. Fund. Appl. Toxicol. 29: 155-167.

Nevertheless, at low exposure concentrations, such as in coal mining, equilibrium will occur. Coal miners will have between 5 and 15 mg of coal dust/g of lung despite long exposures. There are large differences in clearance rates in mammals.

See: Snipes, M B (1989). Long-term retention and clearance of particles in mammalian species. CRC Crit. Rev. Toxicol. 20: Crit Rev Toxicol. 20(3): 175-211.

11-11

CHAPTER 12: FACTORS INFLUENCING THE DOSE FOR GASES AND VAPORS

A. *HIGH WATER SOLUBILITY AND HIGH REACTIVITY*

For these gases and vapors (such as SO_2, HCl, HF, HCHO), calculate like aerosols and assume 100% for α.

They are essentially removed by solution *and* reaction at the surfaces of the respiratory tract and are very efficiently scrubbed by the upper respiratory tract.

12-1

There is very little penetration to the alveolar region until higher concentrations are reached and the scrubbing capability of the upper respiratory tract becomes overwhelmed. Many deaths have occurred with accidental high exposures to such gases/vapors. Obviously there is a limit to the scrubbing capability of the upper respiratory tract.

Several gases/vapors have been investigated for regional penetration (e.g., SO_2, several aldehydes). High water solubility alone is not sufficient since acetone or ethanol vapors are not entirely removed by the nose while SO_2 is.

12-2

With SO_2, the reaction with water at pH 7.4 will yield:

$$SO_2 + H_2O \leftrightarrow H_2SO_3 \leftrightarrow H^+ + HSO_3^-$$
$$HSO_3^- \leftrightarrow H^+ + SO3^=$$

Then, HSO_3^- and $SO_3^=$ will react with protein disulfide bonds to yield sulfonates. Similar schemes can be presented for several water soluble and reactive chemicals. In essence, a "sink" is present to retain these types of gases or vapors in the upper respiratory tract.

Note: A lot of research has been conducted on retention of gases on the upper respiratory tract. A good summary of earlier and newer models can be found here: Cohen Hubal, E A, Fedkiw, P S and Kimbell, J S (1996). Mass-transport models to predict toxicity of inhaled gases in the upper respiratory tract. J. Appl. Physiol. 80: 1415-1427.

12-3

1. Importance in toxicological studies

Since mice, rats, guinea pigs, and hamsters are obligatory nose-breathers, the first area affected by these gases will be the nose. Nasal carcinoma in rats and mice vs. monkeys and humans due to formaldehyde is a good example.

See: Casanova, M, Morgan, K T, Steinhagen, W H, Everitt, J L, Popp, J A and Heck, H (1991). Covalent binding of inhaled formaldehyde to DNA in the respiratory tract of rhesus monkeys: pharmacokinetics, rat-to-monkey interspecies scaling and extrapolation to man. Toxicol. Appl. Pharmacol. 17: 409-428.

12-4

2. Extrapolation from small laboratory animals to humans

Toxicological studies of these gases will always underestimate their pulmonary toxicity for humans, assuming that the species in question has the same sensitivity as humans. This is somewhat compensated by the fact that for a given exposure concentration the rodents will receive a higher net dose due to their higher minute ventilation to body weight ratio. However, again deposition sites may differ.

A very difficult problem to solve.

12-5

3. Evaluation of toxicological effects

The nose is a complex organ and several sections must be made in order to evaluate the effects at different levels. It is difficult to predict where lesions will occur but three major epithelial types must be examined: squamous epithelium, respiratory epithelium and olfactory epithelium. Buckley et al. (1984) presented a detailed analysis of lesions in the nasal passages for 10 different chemicals using a standardized procedure. Very few inhalation toxicology studies included histopathological examination of the nose prior to 1980.

See: Buckley, L A, Jiang, X Z , James, R A, Morgan, K T and Barrow, C S (1984). Respiratory tract lesions induced by sensory irritants at the RD50 concentration. Toxicol. Appl. Pharmacol. 74: 417-429.

12-6

B. *LOW WATER SOLUBILITY AND HIGH REACTIVITY*

These will penetrate deeper into the lung, NO_2, O_3, $COCl_2$, TDI, methyl isocyanate (MIC), Cl_2 (although MIC and Cl_2 have "intermediate solubility". They react with pulmonary tissues, generally produce pulmonary edema. They are sometimes called true pulmonary irritants.

Calculate like aerosols, assume 100% for α. Only a few gases have been directly investigated in animals or humans.

12-7

Note: It is always assumed that these chemicals are so reactive that they never, as such, enter the pulmonary capillaries by diffusion across the alveolar capillary membranes. Maybe correct, maybe not. For example, 14CMIC appeared in arterial blood within minutes from the beginning of exposure. This was measured as 14C, not 14CMIC. However, 14CMIC was found to be covalently bound to hemoglobin within the red blood cell. How did it get there? It is possible that MIC could have been transported within the red cells conjugated to a small peptide and released as MIC there to react with hemoglobin, since if reacted with an SH group of something like glutathione or cysteine, this reaction is reversible. Ozone is well-known to induce narcosis in exposed animals. Again, we can present arguments that it does not reach the CNS to exert its effect. Rather, its effect may be due to release of a variety of mediators during the development of pulmonary toxicity. However, we have held the above assumption for too long without testing it directly, but it is not that easy to test.

A few examples of measurements of the uptake of highly reactive vapors are:

Kennedy, A L, Stock, M F, Alarie, Y and Brown, W E (1989). Uptake and distribution of 14C during and following inhalation exposure to radioactive toluene diisocyanate. Toxicol. Appl. Pharmacol. 100: 280-292.

Ferguson, J S, Kennedy, A L, Stock, M F, Brown, W E and Alarie, Y (1988). Uptake and distribution of 14C during and following exposure to [14C] methyl isocyanate. Toxicol. Appl. Pharmacol. 94: 104-117.

12-8

C. *NO (OR VERY LOW) REACTIVITY WITH THE SURFACE OF RESPIRATORY TRACT (APPLICABLE TO ALL SOLVENTS)*

Assume inert gas and no metabolism and no water solubility (none of this is actually true for solvents, but we start from here and then make corrections). The following physical and physiological parameters will influence their uptake from inspired air into blood and clearance out of blood. We consider three compartments:

- **Inspired Air (exposure concentration)**
- **Alveolar Air (concentration in alveolar volume)**
- **Capillary Blood (concentration in blood leaving the lung, i.e., arterial blood)**

12-9

A final equilibrium will be established between all three compartments. However, as shown below, an equilibrium between Alveolar Air and Capillary Blood exists at all times. We now simply need to know how long will it take for an equilibrium to be reached between Inspired Air, Alveolar Air, and Capillary Blood. Many variables need to be taken into account.

1. Physical Parameters (a, b, c)

a. Concentration in Air, as Partial Pressure

The concentration in air (mm Hg) determines the maximum concentration (mm Hg) in blood since equilibrium between the compartments (i.e., inspired air, alveolar air and blood) will be established at some time. Once equilibrium is established, no more can be taken into blood regardless of how long the exposure lasts.

12-10

b. Blood/Air Solubility Coefficient, S

Knowing S gives the maximum concentration in moles or milligrams/Liter of blood which can be achieved at equilibrium. It will be the concentration in air (mg/L) × S. Substances with high S values (methanol, ethanol, acetone, ether, etc.) will take a long time to reach equilibrium between inspired air and blood. Substances with low S values (methane, nitrous oxide, etc.) will reach equilibrium between inspired air and blood very quickly. An S value can be taken for water at 37°C, but be careful (see above tables of S values).

c. Oil/Water Partition Coefficient

This will influence the time to reach equilibrium between inspired air and blood in that if the chemical is highly fat soluble the chemical will be extracted from blood. However, since fatty tissue is poorly perfused, this variable is not important during uptake and can be ignored. It is more important after exposure; it will influence how long it will take to excrete a chemical stored in fatty tissue, particularly hours or days after exposure.

12-11

2. Physiological Parameters (a, b, c, d, e)

a. Minute Ventilation

Important for high S substances. In essence, so much chemical is needed to be transferred into blood to reach equilibrium, that bringing more by increasing minute ventilation will increase the rate at which equilibrium will be reached between inspired air and capillary blood.

b. Cardiac Output

Important for low S substances. In essence, so little chemical is needed to be transferred into blood to establish equilibrium that exposing blood faster to alveolar air (higher cardiac output) will decrease the time needed to reach equilibrium. Cardiac output is also called "perfusion" as far as the lung is concerned, so low S substances will be "perfusion-limited". Increased perfusion decreases the time to reach equilibrium.

12-12

c. Diffusion Capacity
Since vapors must move across the alveolar-capillary membrane, a limitation due to diffusion may occur. This is not so for industrial solvents. There is sufficient time for equilibrium to occur between the two compartments. Therefore, equilibrium is assumed to exist *at all times* between *alveolar air* and *capillary blood*. The only "diffusion-limited" gas is CO.*

d. Amount of Blood and Body Water To Be Saturated:
5 liters of blood and total body water, 40 liters

e. Amount of Fatty Tissue To Be Saturated:
~15% body weight

*Note: See Chapter 19 for the details with this gas.

12-13

f. Biotransformation or Metabolism
To the extent that a chemical is metabolized, this will reduce its amount in blood and therefore uptake will continue. Usually this is via an enzymatic saturable pathway and will be important at low concentrations. At high concentrations, the amount of a chemical that is metabolized will be too low to influence the equilibrium concentration in blood.

How equilibrium is established:
The driving force is the partial pressure difference (mm Hg) between two phases.

Beginning	Equilibrium
100 mm Hg AIR (ALVEOLAR) ↓↓↓↓	100 mm Hg AIR
0 mm Hg BLOOD (PULMONARY CAPILLARIES)	100 mm Hg BLOOD

12-14

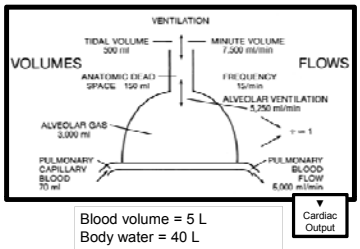
Low solubility coefficient in example below:
Few moles needed in blood to reach equilibrium with air

Beginning	Equilibrium
Moles oooooooooooooooo oooooooooooooooo AIR	Moles oooooooooooooooo oooooooooooooooo AIR
BLOOD	o BLOOD

High solubility coefficient in example below:
Large molar concentration needed in blood to reach equilibrium with air

Beginning	Equilibrium
Moles oooooooooooooooo oooooooooooooooo AIR	Moles oooooooooooooooo oooooooooooooooo AIR
BLOOD	oooooooooooooooo oooooooooooooooo oooooooooooooooo oooooooooooooooo BLOOD

12-15



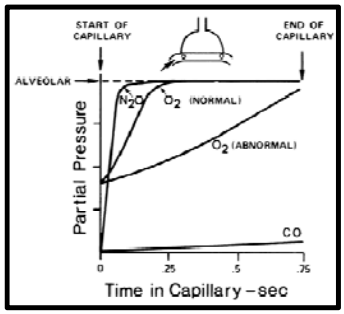
Blood volume = 5 L
Body water = 40 L
Body fat = 15 L
Loss due to biotransformation

When exposure starts, the concentration in the first breath will drop due to dilution in alveolar gas, but equilibrium will be achieved between the alveolar gas and the capillary blood. This will continue until the partial pressure is equal in all 3 compartments. The loss due to biotransformation will be replenished, as for O_2 below.

Note: Useful diagrams (12-16 and 12-17) to understand gas uptake.
Modified from West, J B (2011).
Respiratory Physiology: The Essentials (Ninth Edition).
Reprinted with permission from Williams and Wilkins.

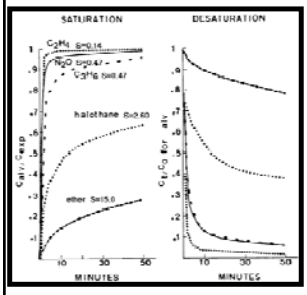
12-16

In this diagram, you see that there is sufficient time for equilibrium to be established between the alveolar air and the capillary blood (i.e., venous blood, coming back and leaving as arterial blood).



From West (2011). Uptake of CO, N_2O, and O_2 along the pulmonary capillary. Note that the blood partial pressure of N_2O virtually reaches that of the alveolar gas very early in the capillary so that the transfer of this gas is "perfusion-limited". By contrast, the partial pressure of CO in the blood is almost unchanged so that its transfer is "diffusion-limited". O_2 transfer can be either "perfusion-limited" or "diffusion-limited" depending upon the conditions.

12-17



This figure shows the influence of S on the time to reach equilibrium during an exposure and the time to release after an exposure. The Y axis is given as: $C_{alveolar}$ / $C_{expired\ air}$ which is the same as C_{blood} / $C_{inspired\ air}$ since $C_{alveolar}$ is at equilibrium with C_{blood} when C is given in mm Hg partial pressure. Original from Eger, adapted by Fiserova-Bergerova and re-modified. S value is the same for nitrous oxide and for cyclopropane. The small difference is due to a higher exposure concentration with nitrous oxide.

12-18

Summary of Factors Influencing Uptake and Equilibrium[a]

Agents	S	Concentration Effect	Time to Reach Equilibrium	Quantity in Blood in mg at Equilibrium	Physiologic Factors Influencing Uptake and Time To Reach Equilibrium
Ethanol	1000	Important	Slow	Large	Ventilation
Acetone	250	↓	↓	↓	↓
Ethyl Ether	15	↓	↓	↓	↓
Benzene	8	↓	↓	↓	↓
Chloroform	7	↓	↓	↓	↓
Halothane	2.5	↓	↓	↓	↓
X	1	↓	↓	↓	**50/50**

[a]Adapted from Goldstein, A, Aronow, L and Kalman, S M (1974). Principles of Drug Action. Wiley, NY, pp. 338-353.

12-19

Summary of Factors Influencing Uptake and Equilibrium[a] …. continued

Agents	S	Concentration Effect	Time to Reach Equilibrium	Quantity in Blood in mg at Equilibrium	Physiologic Factors Influencing Uptake and Time To Reach Equilibrium
Acetylene	0.8	↓	↓	↓	↑
Nitrous Oxide	0.5	↓	↓	↓	↑
Ethylene	0.14	↓	↓	↓	↑
Sulfur Hexafluoride	0.006	Not important	Fast	Low	Cardiac output

12-20

D. ***PRACTICAL USE OF THE ABOVE CONCEPTS IN TOXICOLOGY***

1. Concentration effect

The exposure concentration is important for substances of high S values (ethanol, for example) because they have substantial solubility in water and therefore a substantial amount will be scrubbed by all surfaces of the respiratory tract above the alveolar level and much less will be received at the alveolar level. Thus at low "environmental exposures, ppb or low ppm", there is no possibility for an equilibrium to exist between inspired air and capillary blood.

12-21

Also, at low concentrations, biotransformation is effective and again will prevent equilibrium from being achieved. Thus, PBPK-dosimetry models, relying on the above principles, are no longer appropriate.

What do you do? Calculate as an aerosol, assuming 100% retention. This will at least give you a ballpark figure for how much has been inhaled.

2. Alcohol breath test

Alveolar air x S = Blood concentration
(mg/100 mL) (mg/100 mL)

http://www.ctvnews.ca/canada/new-alberta-drunk-driving-penalties-take-effect-1.939005

12-22

Of obvious forensic importance and relies upon the fact that alveolar air and capillary blood are at equilibrium. With breathalyzer machines, the S value used for ethanol is 1756. S values between 1000 and 1756 have been used for ethanol, depending upon how various tests are made.

Note: Some interesting articles to read about this test.

Haagard, H W, Greenberg, L A , Miller, D P , and Carroll, R P (1941). The alcohol of the lung air as an index of alcohol in the blood. J. Lab. Clin. Med. 26: 1527-1541.

Jones, A W (1983). Determination of liquid/air partition coefficients for dilute solutions of ethanol in water, whole blood and plasma. J. Anal. Toxicol. 7: 193-197.

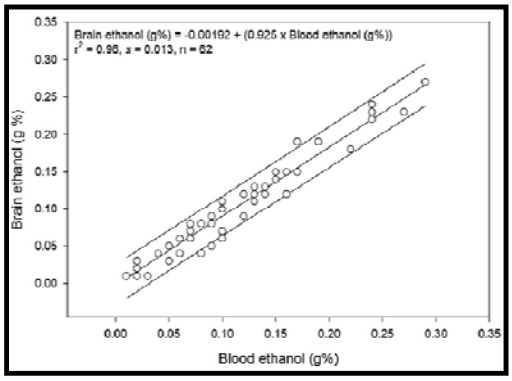
Ohlsson, J, Ralph, D D, Mandelkorn, M A, Babb, A L and Hlastala, M P (1990). Accurate measurement of blood alcohol concentration with isothermal rebreathing. J. Stud. Alcohol 51(1): 6-13.

12-23

3. What about ethanol in brain tissue?

Since the effect of ethanol is at the CNS level, can we relate brain ethanol to blood ethanol and alveolar air ethanol? The answer is yes, and convenient for analysis in dead victims when obviously alveolar air is not available and even blood may not be available. The brain receives 25% of cardiac output, so blood/brain transfer is very fast for low MW chemicals, regardless of high or low S values. The fact that there is a perfect correlation between blood and brain as shown below also indicates that brain tissue is "water", not fat.

12-24



From: Alarie, Y (2002). Toxicity of fire smoke. Crit. Rev. Toxicol. 32: 259-289.

12-25

4. Monitoring exposure to solvents with alveolar air sampling.

Alveolar air samples concentration (ppm) of humans exposed to trichloroethylene at 200 ppm, 7 hours/day for 5 days.

	1st Day	2nd Day	3rd Day	4th Day	5th Day
Pre-Exposure	0.01	1.2	1.6	1.6	1.6
3 hrs	76	75	76	79	76
30 min After Lunch	10.3	10.9	11.5	8.4	8.5
1 hr Post-Exposure	8.3	9.4	9.0	7.7	8.7
3 hrs Post-Exposure	5.1	4.4	3.5	3.5	3.6
6 hrs Post-Exposure	3.3	2.8	2.4	2.9	2.9

Note: Does not reach equilibrium with inspired air. S is moderately high (9.2) and thus equilibrium between inspired air and blood will be established slowly, but also trichloroethylene is biotransformed to trichloroacetic acid (TCA) and trichloroethanol (TCE) which continuously reduces the amount of trichloroethylene in blood. Adapted from Stewart, R D, Dodd, H C, Gay, H H and Erley, D S (1970). Experimental human exposure to trichloroethylene. Arch. Environ. Health 20:64-71.

12-26

Yes, it is important how the breathing maneuver is done and how the gas (air) sample is collected.

Note: Alveolar air is best obtained by asking the person to breathe out into a long tube. The air first exhaled is really coming from the conducting airways (dead space), not in contact with capillary blood. From a tap in the tube close to the mouth, a sample of air is taken toward the end of exhalation and this sample will truly represent alveolar air. A more convenient way is to have the person breathe out through a short tube and capping the tube (both ends) at the end of exhalation. The tube can then be shipped for analysis. Exhaling into a bag is OK; there will be some dilution from the conducting airways air (i.e., dead space volume), but it will be close enough. The proper term for this sample would be "mixed-exhaled air".

See Stewart, R D, Hake, C L and Peterson, J E (1974). Use of breath analysis to monitor trichloroethylene exposures. Arch. Environ. Health 29: 6-13.

12-27

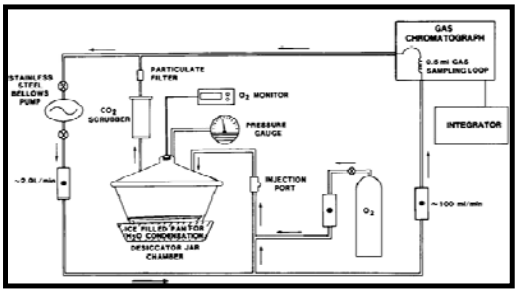
5. Is a chemical vapor biotransformed and if so, at what rate?

a. In a closed chamber, the concentration of an inert and not metabolized (biotransformed) gas or vapor will decrease due to absorption in blood and tissues in the animal or human located in the chamber and then remain stable as equilibrium between inspired air and blood is reached.

b. If a chemical is biotransformed, equilibrium will not be reached, reduction of its concentration in the chamber will continue.

c. The rate at which the concentration decreases is the biotransformation rate. This was done a long time ago, demonstrating that oxygen is metabolized! The rate at which oxygen decreases is simply oxygen consumption. Same is true for an organic solvent and this can be measured with modern analytical instrumentation as shown in the figures on the next pages.

12-28



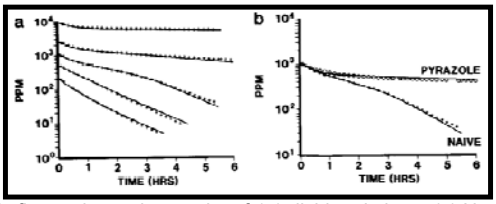
From: Gargas, M L, Andersen, M E and Clewell, H J III (1986). A physiologically based simulation approach for determining metabolic constants from gas uptake data. Toxicol. Appl. Pharmacol. 86: 341- 352.
Reprinted with permission from Academic Press.

12-29

The figure on 12-29 shows a closed chamber system for measuring the uptake of gases or vapors. This is the same system that is used for measuring oxygen uptake or oxygen consumption, except that a gas chromatograph or another suitable analyzer is used to monitor the concentration of the gas to be studied. O_2 is replenished in this system and CO_2 is scrubbed to maintain a normal air atmosphere.

The same can be done with human subjects, using a face mask to make a closed system.

12-30



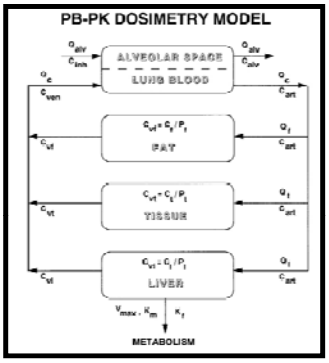
This figure shows the uptake of 1,1-dichlorothylene. (a) Naïve rats were exposed at initial chamber concentrations of 200, 550, 1200, 2700, and 10,000 ppm. The smooth curves were generated by the computer model after a visual optimization process to obtain the kinetic constants which gave the best representation of the set of curves (Table 2). (b) A comparison of uptake at an initial chamber concentration of 1200 ppm in pyrazole-treated and naïve rats. The kinetic constants used for pyrazole are in Table 2. Exposures were conducted with three rats in a 9.1-liter chamber.

12-31

The figure on 12-31 shows the uptake of dichloroethylene in a closed chamber. At high concentrations, the effect of biotransformation is hardly seen. The curves show a decline that can be attributed to equilibrium between chamber air and whole animal. At lower concentrations, there is a clear, continuous decline in chamber concentration and the slope of the curves is the biotransformation rate. Further proof of biotransformation is by using a metabolic inhibitor, pyrazole.

Figure on 12-31 from: Gargas, M L, Andersen, M E and Clewell, H J III (1986). A physiologically based simulation approach for determining metabolic constants from gas uptake data. Toxicol. Appl. Pharmacol. 86: 341-352. Reprinted with permission from Academic Press.

12-32



Summary of components and principles necessary for physiologically based pharmacokinetic (PB-PK) modeling of uptake, equilibrium and excretion of inhaled vapors. From: Gargas, M L (1991). CIIT Activities, Vol. 11, No.3. Reprinted with permission from CIIT.

12-33

The figure on 12-33 presents a typical PB-PK dosimetry model, consisting of three tissue compartments and a gas-exchange lung compartment. Flows Q are alveolar (alv), total cardiac (c), and those to the fat, liver, and other tissues (f, l, and t, respectively). Concentrations C are those in the inhaled air (inh), alveolar air (alv), arterial blood (art), venous blood (ven), and in the venous blood leaving the tissues (vf, vl, and vt). The venous blood concentrations are dependent on the chemical concentrations in the tissues and the tissue:blood partition coefficients (P_i), Metabolic removal of chemical from the liver of this model is described kinetically by either a maximum rate, V_{max}, and the Michaelis constant, Km, and/or a first-order rate constant, K_i.

12-34

6. Laboratory Animals vs. Humans.

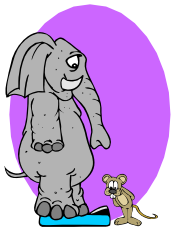
It is important to remember that small laboratory animals have higher minute ventilation to body weight ratio than humans. Therefore, if a mouse and a human are exposed at the same concentration, equilibrium (or any blood concentration) will be reached faster in the mouse than he human.

This principle was used with a canary or mouse in mines, to monitor carbon monoxide (CO). When the canary falls off its perch due to high COHb (around 40-50%), the human still has time to escape. I would not try this, but in any event it illustrates the principle. The canary is not more sensitive than humans; it simply reaches 40-50% COHb a lot faster than a human will.

12-35

We can turn this around with elephants that are coal mining. They can take a woman with them, and when the woman falls off her perch, the elephants will know to start getting out.

OK, OK … they can also take a man with them, but it would not be as good!



12-36

Now, would this work to warn about high methane concentrations in mines? Absolutely not. No way. Methane has a low S value and therefore the time difference to displace oxygen in blood between a canary and a woman is just too small. Remember, the lower S is, the faster equilibrium is established and the smaller the difference will be between a small and large animal. It works for CO because this gas is "diffusion- limited", the equivalent of a very high S value.

The second item to remember is that small laboratory animals have a higher metabolic rate than humans. Thus, if a metabolite of the gas or vapor is the active toxic species, more of it will be produced than in humans even though both are exposed to the same concentration. Here, PB-PK and other modeling techniques are very useful.

12-37

Note: Here are a few more references on this topic of solvent uptake. There are so many available now that it is difficult to give a comprehensive list. But those listed below illustrate the basic principles.

Fiserova-Bergerova, V (1983). Modeling of inhalation exposure to vapors: Uptake, Distribution and Elimination. Volumes I and II. CRC Press, Boca Raton, FL.

Sato, A, Endoh, K, Kaneko, T and Johanson, G (1991). A simulation study of physiological factors affecting pharmacokinetic behaviour of organic solvent vapours. Brit. J. Indust. Med. 48: 342-347.

Filser, J G (1992). The closed chamber technique-uptake, endogenous production, excretion, steady-state kinetics and rates of metabolism of gases and vapors. Arch. Toxicol. 66: 1-10.

Andersen, M E, Gargas, M L, Jones, R A and Jenkins, L J Jr (1979). The use of inhalation techniques to assess the kinetic constants of 1,1-dichloroethylene metabolism. Toxicol. Appl. Pharmacol. 47: 385-393.

12-38

CHAPTER 13:
EFFECTS ON THE RESPIRATORY TRACT

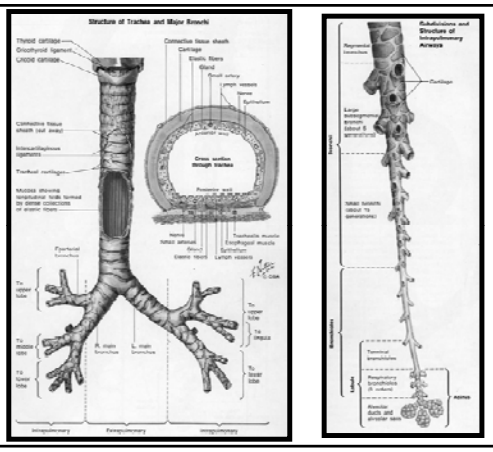
A. *BRIEF REVIEW OF ANATOMY*

Illustrations in this section from: F. H. Netter. The Ciba Collection of Medical Illustrations, Volume 7, Respiratory System. 1980. M.D. Altose. Ciba Clinical Symposia Volume 31, The Physiological Basis of Pulmonary Function Testing, 1979. Ziskind, M.M. Ciba Clinical Symposia Volume 30, Occupational Pulmonary Disease, 1978 and Weiss, E.B. Ciba Clinical Symposia Volume 27, Bronchial Asthma, 1975.

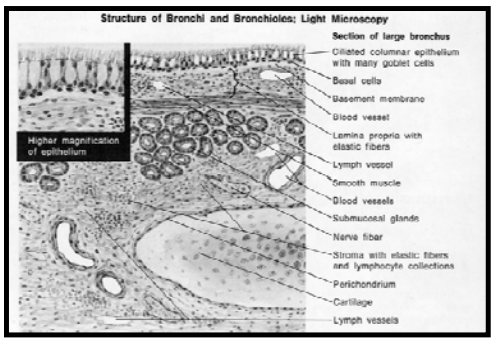
*Top-notch illustrations by Netter in these publications!

Note: All figures in this section reprinted with permission from Ciba Pharmaceutical Company.

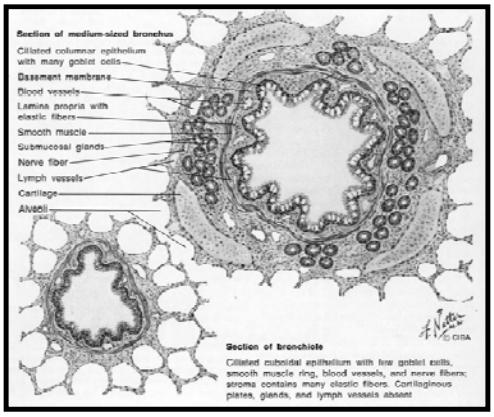
13-1



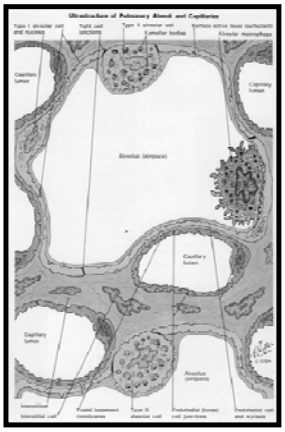
13-2



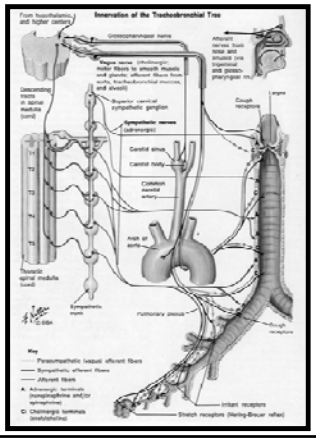
13-3



13-4



13-5



13-6

The respiratory tract is well-innervated, from the tip of the nose to the alveoli.

- Stimulation of trigeminal nerve endings in nasal mucosa results in a reflex delay for expiration to take place and a net decrease in respiratory frequency.
- Cough receptors in larynx, most sensitive area to elicit coughing by irritant gases or aerosols.
- At the alveolar level, Type J receptors (not shown in above diagram) are unmyelinated C-fibers which are stimulated by pulmonary irritants creating inflammation and edema. This stimulation results in rapid shallow breathing which is then followed by the addition of a pause at the end of expiration if stimulation is more intense.

13-7

B. *ACUTE EFFECTS*

A practical way to classify airborne chemicals is by taking the first level of the respiratory tract at which they act, as the exposure concentration increases from zero (see 13-10 for references).

1. Sensory Irritants

a. Definition

Chemicals, which when inhaled via the nose, will stimulate trigeminal nerve endings, evoke a burning sensation of the nasal passages and inhibit respiration. Also will induce coughing from laryngeal stimulation and lachrymation from corneal stimulation.

13-8

b. Other Characteristics

At high concentration, particularly on moist facial skin, they will induce a burning sensation. Some have odor and taste (SO_2). Many will induce airways constriction, usually at higher concentrations.

c. Other Terms Used to Describe Their Action

Upper respiratory tract (URT) irritant, nasal or corneal stimulant, common chemical sense stimulant, chemogenic pain stimulant, suffocant, lachrymator, tear gas, sternutator, "eye, nose, and throat" irritant.

13-9

d. Typical examples

Chloracetophenone, o-chlorobenzylidene malononitride, β-nitrostyrene, diphenyl-aminoarsine, sulfur dioxide, ammonia, acrolein, formaldehyde, capsaicin, smoke from burning materials.

Earlier reviews:

Alarie, Y (1973). Sensory irritation by airborne chemicals. CRC Crit. Rev. Toxicol. 2: 299-363.

Nielsen, G D (1991). Mechanisms of activation of the sensory irritant receptor by airborne chemicals. CRC Crit. Rev. Toxicol. 21: 183-208.

More recent review:

Alarie Y, Nielsen G D and Schaper M (2000). Animal bioassays for evaluation of indoor air quality. *In: Indoor Air Quality Handbook*. Spengler, J D, Samet, J M and McCarthy, J F (Eds), McGraw-Hill, NY, pages 23.1-23.49.

13-10

2. Bronchoconstrictors (Airways Constrictors)
a. Definition
They act primarily on the conducting airways and should probably be called "airways constrictors". They may act on the larger or smaller airways causing their constriction and as a result will increase resistance to airflow into and out of the lung (increase in airway resistance). If acting on the smaller airways, some regions of the lungs may be closed to ventilation, resulting in air trapping in the lungs, and a decrease in dynamic lung compliance will result.

13-11

b. Mechanisms
Their action may be via a direct effect on airway smooth muscles, by axonal reflex, vago-vagal reflexes following stimulation of vagal nerve endings, by liberation of histamine or other mediators.

c. Other Effects
Increase mucus secretions, induce inflammatory reaction

d. Typical examples
Histamine and cholinergic agonists, sulfur dioxide, following sensitization by allergens such as foreign proteins or chemicals acting as haptens (toluene diisocyanate, trimellitic anhydride, … see below).

13-12

3. Pulmonary irritants
a. Definition
Chemicals, which when inhaled, will stimulate sensory receptors within the lungs resulting in an increase in respiratory rate and decrease in tidal volume (rapid shallow breathing, RBS). This results in a sensation of dyspnea or breathlessness. This RBS is observed in humans, cats, rabbits, dogs, guinea pigs, but less in mice or rats (see below). With more intense stimulation, the breathing pattern changes to include an apneic period (i.e., "pause") between each breath. As the duration of this pause increases, a decrease in respiratory frequency (f) is observed.

13-13

This apneic period is very easily detected in mice (see below), but the rapid and shallow breathing phase is usually not very pronounced in this species.

b. Other characteristics
These chemicals are capable of inducing pulmonary edema which will develop slowly (a few hours). They have no or little action as sensory irritants of the eye or nasal passages at concentrations capable of inducing pulmonary irritation.

Therefore, they provide *little warning of their presence*. *Very hazardous*.

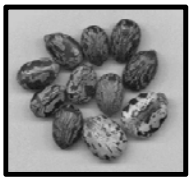
13-14

Some have odorant quality (e.g., O_3), but this fades quickly. Some will also induce bronchoconstriction at higher concentrations.

c. Other terms to describe their action
Lower respiratory tract irritants, lung irritant, deep lung irritant, edemagenic agents

d. Typical examples
Ozone, nitrogen dioxide, sulfuric acid mist, phosgene, sulfur mustard, lewisite, sulfur pentafluoride, cadmium fumes, paraquat aerosol, hexamethylene diisocyanate (HDI)-trimer aerosol, cotton dust, endotoxin aerosol

13-15



A glycoprotein (ricin), extracted from ricin beans (also called castor beans), is an extremely potent pulmonary irritant. A few ng inhaled (or injected) will induce massive pulmonary edema in a few days, and it is also effective if ingested. A favorite in spy stories because it leaves no traces, and now of the Homeland Security.

13-16

4. Respiratory irritants
a. Definition
Chemicals, which when inhaled, can act as a sensory irritant, a bronchoconstrictor and a pulmonary irritant. These chemicals are capable of all three actions and there is little difference between the concentrations at which they induce an effect at all these levels (i.e., nose, conducting airways, deep lung).

b. Typical examples
Chlorine, ketene, chloropicrin, dichloromethyl ether, chlorine pentafluoride, diepoxybutane, methyl isocyanate

13-17

Note: While the above classifications "by first level of action" are practical, it should be kept in mind that some agents have been better studied than others. Also, the effect can be time-dependent. For example, the sensory irritating effect of methyl isocyanate (MIC) is immediate and MIC is a potent sensory irritant. This effect is followed by an airway constricting effect, airway inflammation and hemorrhage and pulmonary edema and pulmonary hemorrhage. Thus, classifying MIC as a "sensory irritant" would clearly underestimate its potential toxicity to the lower respiratory tract.

13-18

The reverse would be true for formaldehyde, a potent sensory irritant. Yet, it has little bronchoconstricting effect in humans (even asthmatics) and no pulmonary irritating effect. Thus, classifying it as a sensory irritant is more appropriate. We can then look at sulfur dioxide, not particularly potent as a sensory irritant, but it will induce bronchoconstriction in humans, particularly asthmatics. Thus, it is probably better to look at this chemical as a bronchoconstrictor rather than a sensory irritant.

So, take this classification as a starting point!

13-19

CHAPTER 14:
HOW TO EVALUATE SENSORY IRRITATION, BRONCHOCONSTRICTION, AND PULMONARY IRRITATION

A. *BRONCHOCONSTRICTION*
It is easier to start with bronchoconstriction. Once this is understood, we can move to a method to measure any of the above.

When bronchoconstriction occurs, the diameter of the airways decreases. This will increase the resistance to airflow in and out (but particularly out) of the lung. More pressure will be needed to move air in and out of the lung.

14-1

Since resistance is pressure/airflow (cm H_2O/ml/sec), more pressure will be needed to produce the same airflow. The principles and measurements were published by German physiologists in the 1920s, but were used in toxicology starting only by mid-1950s. Using small animals, the guinea pig is a good choice because it has extensive bronchial musculature and bronchoconstriction can be so intense that the animal will die.

In order to measure resistance to airflow (new nomenclature is conductance, or simply 1/resistance), we need to measure three variables:

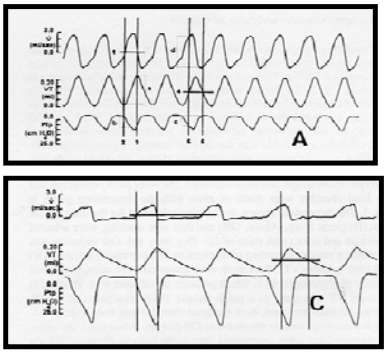
14-2

- **Airflow (Inspiratory and expiratory airflow, i.e., air in and out of the lung with each breath), measured by a pneumotachograph (see above).**
- **Tidal volume (amount of air in and out of the lung with each breath), obtained by integration of airflow measurement.**
- **Transpulmonary pressure (the pressure difference between alveolar pressure and mouth pressure), measured by inserting a catheter tip transducer into the thoracic cavity of the animal.**

Note: When measuring these variables, we can also calculate the dynamic compliance of the lung (CdynL) (i.e., how stiff the lung is). Compliance is volume/pressure (ml/cm H_2O); new nomenclature is elastance, or simply 1/compliance.

14-3

This figure shows how airways resistance (RL) and dynamic compliance (CdynL) are measured*.



14-4

The figure on 14-4 presents oscillographic tracings of airflow ($\dot{V}$), tidal volume (VT), and transpulmonary pressure (Ptp), as obtained in an unanesthetized mouse:

Panel A, during normal air breathing and
Panel C, during severe bronchoconstriction during inhalation of carbamylcholine aerosol

The figure on 14-4 is from: Vijayaraghavan, R, Schaper, M, Thompson, R, Stock, MF, and Alarie, Y (1993). Characteristic modifications of the breathing pattern of mice to evaluate the effects of airborne chemicals on the respiratory tract. Arch. Toxicol. 67: 478-490.

The first article using the measurements of CdynL and RL in toxicology was published by Amdur, MO and Mead, J (1955). A method for studying the mechanical properties of the lungs of unanesthetized aninals: application to the study of respiratory irritants. Proc. 3rd Natl. Air Pollution Symposium, p.150. Many irritants were investigated (sulfur dioxide, formaldehyde, acrolein, etc.) and a summary of this work presenting their potency can be found in the 1973 review by Alarie quoted above.

14-5

Panel A: A horizontal line (Line 1) is drawn at zero airflow to separate inspiration (upward) from expiration (downward). Vertical lines (Lines 2 and 3) are drawn at the beginning and end of expiration, respectively (i.e., from points of zero flow). A horizontal line (Line 4) is drawn at 0.5 VT and vertical lines (Lines 5 and 6) are drawn at 0.5 VT.

Then, CdynL is calculated from the ratio of the amplitude of a divided by the amplitude of b. That is, the amount of air inhaled (ml) divided by the pressure required (cm H_2O) from the beginning to end of inspiration, these being determined from zero flow points, from Line 1.

14-6

Then, RL is calculated from the ratio of the amplitude of c divided by the amplitude of d. That is, the airflow (ml/sec) from mid-tidal volume during inspiration and expiration divided by the pressure difference (cm H_2O) at mid-tidal volume. Under these conditions, RL= 2.31 ml/cm H_2O/sec and CdynL = 0.021 ml/cm H_2O.

Panel C: Same lines are drawn. RL now increased to 20.19 and CdynL decreased to 0.005.

What is also important is to look at the breathing pattern. During bronchoconstriction, the breathing pattern is drastically changed (admittedly exaggerated here because of a severe effect).

14-7

But, it illustrates several important points:

1. **The duration of inspiration is longer and the airflow during inspiration is lower, but VT is just about the same (longer duration at lower airflow = same volume). So, the main change during inspiration is a much higher Ptp. Because of this, CdynL decreases. Not entirely because the lung is stiffer, but also because there is so much airways constriction resulting in closure of peripheral airspaces, the inspired air cannot penetrate there.**

---

2. **The duration of expiration is increased dramatically, with a much lower airflow. This much lower airflow, is the major contributor to the increase in RL.**

14-8

3. **Why such a lower airflow during expiration in comparison to almost normal airflow during inspiration? During inspiration, the conducting airways are expanding, despite constriction, airflow can still proceed. During expiration, the airways are collapsing and with constriction, the air cannot move out as well and thus the long duration of expiration at a very low airflow is required.**

---

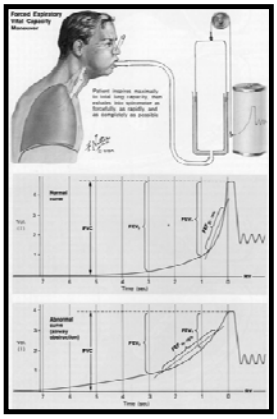
4. **As you can see, if only expiratory airflow is measured, it can indicate bronchoconstriction. This would obviate the need to measure Ptp and simplify things greatly.**

14-9

To measure the potency, exposures are conducted at various concentrations to increase RL up to 200% or so and then the concentration required to increase RL by 100% is calculated.

This permits a valid comparison between different airborne chemicals. The problem, however, is that we have no idea about extrapolating the findings to humans. We take it for granted that if a chemical is positive in guinea pigs it will be so in humans and we take it for granted that the potency will be the same. Not unusual to do this in toxicology.

14-10



RL has also been used to measured airways constriction in humans (bronchoconstriction), but has been replaced by the measurement of airflow during a forced expiration after a maximal inspiration.

FEV1 is the volume of air exhaled during the first second after a maximum inspiration.

14-11

The test can be performed before and after inhalation of an agent. Usually a methacholine aerosol is used, with increasing concentrations, to create a decrease in FEV1 of 20%. This is called the provocation dose to decrease FEV1 by 20% (PD20) and is used to assess the sensitivity of the airways.

The FEV1 test is widely used in clinical practice and in epidemiological studies and will detect obstruction of the airways due to bronchoconstriction, inflammatory reactions of the airways walls, accumulation of mucus secretions, etc.

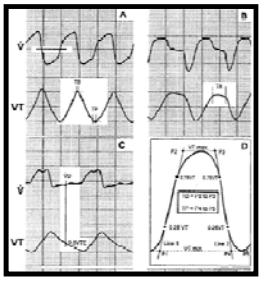
14-12

B. *A GENERAL APPROACH*

With a mouse held in a body plethysmograph and measuring inspiratory and expiratory airflow, we get the following oscillographic tracings under normal conditions and characteristic changes when the mouse is exposed to a sensory irritant, a bronchoconstrictor, or a pulmonary irritant.

The idea is to recognize the characteristic change(s), to quantitate them, and then to develop a concentration-response relationship to estimate potency.

14-13

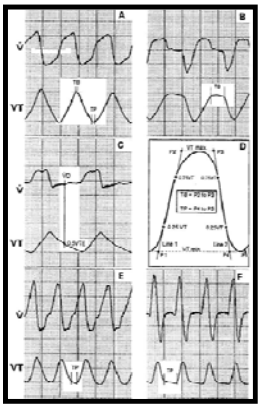


A: Normal conditions
Airflow signal ($\dot{V}$) and integrated $\dot{V}$ to yield VT. A horizontal line is drawn at zero $\dot{V}$, separating airflow during inspiration ($\dot{V}I$), upward and airflow during expiration ($\dot{V}E$), downward.

D: Measuring TB and TP
Hand-drawn wave showing how TB (duration of braking) and TP (duration of pause) are measured.

Note: This figure (shown above and on 14-15, 14-16) is taken from:
Vijayaraghavan, R, Schaper, M, Thompson, R, Stock, MF, Boylstein, LA, Luo, JE and Alarie, Y. (1994). Computer assisted recognition and quantitation of the effects of airborne chemicals acting at different areas of the respiratory tract in mice. Arch. Toxicol. 68: 490-499.

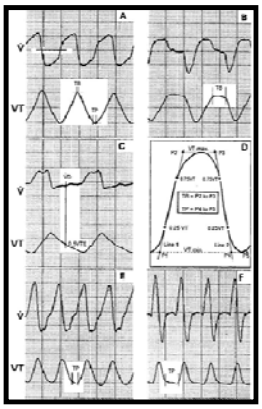
14-14



B: During sensory irritation
Note the major change: an increase in TB. This will also result in a net decrease in respiratory frequency (f).

C: During bronchoconstriction
Note the major changes: longer duration of expiration with much lower $\dot{V}$ than normal. To quantify this change, $\dot{V}$ at mid-tidal volume during expiration (0.5 VTE), abbreviated $\dot{V}D$ here is measured. $\dot{V}D$ has also been abbreviated EF50, expiratory flow at 0.5 VT. There is also a net decrease in f.

14-15



E: During pulmonary irritation
Note the major changes: VT is lower and f is higher (rapid, shallow breathing) and there is a small increase in TP. This is called the first phase of pulmonary irritation (P1).

F: During pulmonary irritation
Note the major changes: the rapid shallow breathing increases and the duration of TP increases to an apneic pause between breaths. TP will continue to increase and will result in a net decrease in f. This is called pulmonary irritation (P).

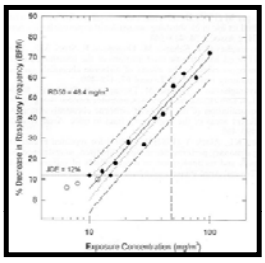
14-16

C. *SENSORY IRRITATION: MEASURING POTENCY*

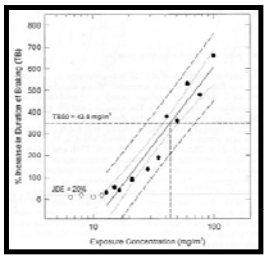
Given the above, we can assess whether or not an airborne chemical has sensory irritating properties by measuring TB or by simply measuring respiratory frequency (f or BPM) provided that the decrease in f is due to an increase in TB, obviously.

Note
The figures shown on 14-18 were taken from:
Alarie, Y (1998). Computer-based bioassay for evaluation of sensory irritation of airborne chemicals and its limit of detection. Arch. Toxicol. 72: 277-282.

14-17



Concentration-response relationship for the breathing frequency variable (BPM) during exposure to CBC. The regression line (*solid*), 95% CI (*dotted lines*), and 95% PI (*dashed lines*) are presented. *Open circles* indicate data points omitted, see text. The regression equation is %BPM = 59.51 + (64.99 x log concentration), $r^2 = 0.96$.



Concentration-response relationship for the duration of braking variable (TB) during exposure to CBC. The regression line (*solid*), 95% CI (*dotted lines*), and 95% PI (*dashed lines*) are presented. *Open circles* indicate data points omitted, see text. The regression equation is %TB = 824.9 + (715 x log concentration), $r^2 = 0.93$.

14-18

Notes:
In the figures on 14-18, the maximum increase in TB that can occur is about 700%. Therefore, 350% is taken as the mid-point and TB50 = 43.8 mg/m3. For BPM, the maximum decrease is 100%, and 50% is taken as the mid-point. Thus, the concentration necessary to decrease respiratory frequency by 50% (RD50) = 48.4 mg.

In the figures on 14-18, JDE = Just Detectable Effect. Or what an analytical chemist would call, "Limit of Detection". DO NOT INCLUDE THE DATA POINTS BELOW THE JDE IN REGRESSION ANALYSIS. Every bioassay has a LIMIT OF DETECTION. A fact ignored in toxicology, the result being that we get J, U, and hockey stick curves, and silly notions like hormesis. Hormesis is snake oil, taking advantage of the fact that toxicologists do not establish a limit of detection for their bioassays.

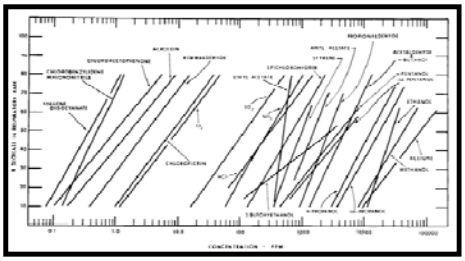
14-19

There is no denying that establishing a detection limit is dependent on many factors (statistical, as well as practical), but it needs to be established. Otherwise, picking data points at the low end of the response level is complete nonsense. If you don't have a detection limit, stick with potency, estimated at the mid-point of the response range. There is a good reason why statisticians selected this mid-point in original bioassays and this practice should not be changed. Unfortunately, it has been changed with all kind of nonsense such as hormesis and the NOEL. Merry Christmas. Do you believe in Santa? This is what NOEL is, no more and no less. The same is true for the others such as NOAEL, AEL, etc., etc. There is no scientific basis for such. Complete nonsense, taking advantage of the fact that toxicologists do not establish a limit of detection for their bioassays. An analytical chemist would be killed for not giving you a limit of detection; toxicologists do this with impunity.

14-20

D. *SENSORY IRRITATION:*
COMPARING POTENCY IN MICE

A wide variety of sensory irritants have been evaluated and their potency can be compared from the concentration-response curves, as presented below.



14-21

The figure on 14-21* presents concentration-response curves for sensory irritants, from very potent (highly reactive chemicals) to very weak (mostly solvents).

These concentration-response curves were obtained using the original bioassay**, rather than the computerized one described above. At that time, a pressure transducer was attached to the plethysmograph to measure tidal volume instead of using a pneumotachograph at described above. Thus the characteristic change to the tidal volume wave was different and the method could detect only sensory or pulmonary irritation, but not bronchoconstriction since airflow was not measured.

*Alarie, Y (1981). Dose-response analysis in animal studies: prediction of human responses. Env. Health Perspectives 42: 9-13.

**Alarie, Y (1966). Irritating properties of airborne materials to the upper respiratory tract. Arch. Environ. Health 13: 433-449.

14-22

A list of chemicals and mixtures investigated for sensory or pulmonary irritation can be found in:

Schaper, M (1993). Development of a database for sensory irritants and its use in establishing occupational exposure limits. Am. Ind. Hyg. Assoc. J. 54: 488-544.

ABSTRACT

A database was developed for chemicals whose sensory-irritating properties had been investigated using a previously described animal bioassay. In this bioassay, mice were exposed to an airborne chemical, and changes in their respiratory pattern were determined. For each chemical tested, the concentration capable of producing a 50% decrease in respiratory rate (RD50) was obtained and its relative potency estimated. For the current study, 295 such airborne materials, including single chemicals and mixtures, were found in the literature. A total of 154 RD50 values were obtained in male mice of various strains for the 89 chemicals in the database for which there were also TLVs. An examination of the TLVs and RD50 values demonstrated, as previously with the smaller dataset (n = 40), a high correlation (R2 = 0.78) of the TLVs with 0.03 x RD50. This supports the continued use of the animal bioassay for establishing exposure limits to prevent sensory irritation in the workplace. No other bioassay provides this type of information or has been used so extensively to suggest guidelines for occupational exposures.

An updated list can be found at: www.yvesalarie.com

14-23

E. *SENSORY IRRITATION: EXTRAPOLATION TO HUMANS*

1. Qualitative

Any bioassay must be validated. Validation refers to correct predictions (correlation) of positive and negative findings between the qualitative response obtained in the bioassay and the qualitative response obtained in humans. It is not necessary that the type of response be the same in both; we only need for one to predict the other. A formal validation was undertaken for sensory irritation by evaluating 51 chemicals in mice and humans. The results showed perfect predictions.

14-24

Chemicals inducing the characteristic post-inspiratory change in mice produced sensory irritation in humans (reports of stinging, burning sensation of eye-nose and throat) while those that did not, were found to be nonirritating by humans.

2. Quantitative

One of the goals of industrial and environmental toxicology is to establish safe levels of exposure to chemicals by first assessing their potency for a particular toxic effect, using a validated bioassay. For sensory irritation, the potency is RD50 as given above.

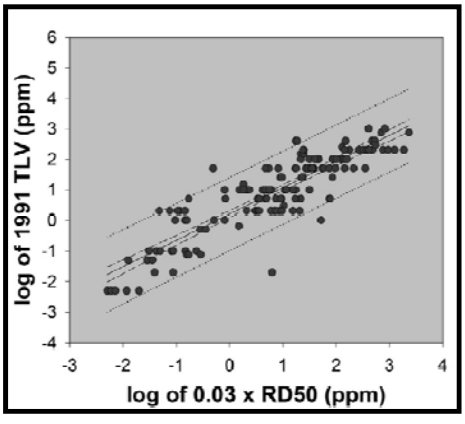
14-25

Thus, quantitative extrapolations to humans have been made as follows:

- At 10 x RD50: severe injury, possibly lethal
- At 1 x RD50: intolerable sensory irritation
- At 0.1 x RD50: some sensory irritation
- At 0.03 x RD50: suggested level for TLV, minor sensory irritation
- At 0.01 x RD50: no sensory irritation
- At 0.001 x RD50: no toxic effect of any kind, acceptable for indoor air quality

For further details on the validation, calibration, and extrapolation to humans, see the following review: Alarie, Y, Schaper, M and Nielsen, DG (2000). Animal bioassays for evaluation of indoor air quality. In: Indoor Air Quality Handbook. Spengler, JD, Samet, JM and McCarthy, JF (Eds). McGraw-Hill, NY, pp 23.1-23.49.

14-26



14-27

The figure on 14-27* presents linear least squares regression analysis for the logarithm of 0.03 x RD50 versus the logarithm of the TLV for 89 chemicals, for which a total of 154 RD50 values are available (several chemicals were tested in different laboratories, thus 154 values instead of 89). The regression line (solid line), 95% confidence interval lines (dashed lines), and the 95% prediction interval lines (dotted lines) are shown. The regression equation is: log TLV (ppm) = 0.202 + (0.86 x log (0.03 x RD50), r^2= 0.78, n = 154 values for 89 chemicals.

*From the Alarie et al. 2000 reference given on page 14-26.

The chemicals used for the above relationship have a TLV primarily based to prevent sensory irritation. Therefore, 0.03 x RD50 is a very good predictor of what the TLV should be for a new chemical or mixture being evaluated.

14-28

The above is true *only if* the RD50 is obtained with male Swiss-Webster mice or strains of mice having a comparable sensitivity since the data points in the above figure were obtained from such. This should be obvious to any toxicologist. Apparently this is not so.

Critics will object, because some strains of mice are more sensitive, or less sensitive. So what? If a more sensitive or less sensitive strain is used, just calibrate again. Instead of 0.03 x RD50, it may be 0.01 or anything else.

And not only this, they add that the data in mice do not correlate with the data in a few rats. As if a rat is just a big male Swiss-Webster mouse! Give me a break.

14-29

And, furthermore, RD50 has no correlation with the Draize test for eye irritation so obviously the system is wrong. How absurd. Why should there be a correlation between nerve endings stimulation (sensory irritation) and tissue destruction?

My secretary is tired of typing the same thing over and over again: RD50 is obtained with male Swiss-Webster mice or with strains of comparable sensitivity and the correlation with TLVs is from these, not rats, guinea pigs or elephants. So, the full answer to these critics is now at:

www.yvesalarie.com

How can "Toxicologists" forget Toxicology 101???

14-30

F. *PULMONARY IRRITATION: MEASUREMENT*

Here you have many choices. All chemicals known to have induced pulmonary irritation in humans will induce rapid shallow breathing in humans, guinea pigs, dogs, monkeys, and rabbits. So, there is no question about the validity of this response in these animals to predict the effects in humans. In all strains of mice tested so far (and to some extent rats, but rats are less sensitive than mice, at least the strains tested so far, so we can forget about them), the rapid shallow breathing will occur first as shown above and in the species noted above.

14-31

Then, TP starts to increase (something not seen in the other species noted above) and therefore TP can be used as an index of pulmonary irritation.

So, make up your mind and use the animal species that you want. Any will do, but just measure the appropriate response.

G. *PULMONARY IRRITATION: EXTRAPOLATION TO HUMANS*

We have already seen above that the test has been validated to predict pulmonary irritation in humans.

14-32

But how is potency, estimated from the increase in f in guinea pigs, for example, or TP in mice as another example, or decrease in respiratory frequency in mice due to an increase in TP related to establishing a TLV for humans?

The only thing we have now is this –
In male Swiss Webster mice, when an exposure concentration results in a 50% decrease in respiratory frequency due to an increase in TP (RD50P), we suggest that RD50P/60 is probably the correct exposure concentration to set a TLV to prevent pulmonary irritation in industrial workers.

14-33

Some recent work by Nielsen et al. with BALB/c mice exposed to the classic pulmonary irritant, ozone, indicates that this strain of mice may be just about as sensitive as humans.

This reference and others on this topic may be found at:

www.yvesalarie.com

Certainly not as good as for sensory irritation, but a good starting point.

14-34

UPDATES ON RD50 AND PULMONARY FUNCTION TESTS

Several recent reviews are available on the use of RD50 in industry and for the general public, as well as for QSARs that can be used to reduce or eliminate the need for animal studies. Also, the nature of the receptor(s) has been reviewed. An excellent review of pulmonary function tests in rodents is listed below.

Luan F, Ma W, Zhang H, Liu M, Hu Z, and Fan BT (2006). Quantitative structure activity relationship models for prediction of sensory irritants (log RD50) of volatile organic chemicals. Chemosphere Volume 63, pp. 1142-1153.

14-35

Nielsen GD, Wolkof P, and Alarie Y (2007). Sensory irritation: Risk assessment approaches. Reg. Tox. and Pharmacol. 48: 6-18.

Gaffney SH and Paustenbach DJ (2007). A proposed approach for setting occupational exposure limits for sensory irritants based on chemosensory models. Ann. Occup. Hyg. 51: 345-356.

Kuwabara Y, Alexeeff GV, Broadwin R, et al. (2007). Evaluation and application of the RD50 for determining acceptable exposure levels of airborne sensory irritants for the general public. Environ. Hlth. Persp. 115(11): 1609-1616.

14-36

Bessac BF, Jordt SE (2008). Breathtaking TRP Channels: TRPA1 and TRPV1 in Airway Chemosensation and Reflex Control. Physiology 23: 360-370.

Saunders, CJ (2013). Dissecting the role of the TRPV1 in detecting trigeminal irritants in three behavioral assays for sensory irritation. F1000 Res 2: 74.
Published on-line at doi:
http://f1000research.com/articles/2-74/v1

14-37

Heinz-Gerd Hoymann (2012). Lung function measurements in rodents in safety pharmacology studies. Frontiers in Pharmacology 3: 1-11.

This article is the best review of all methods used in rodents to evaluate effects on the respiratory tract. You can download the pdf file of the full article from:

fphar.2012.00156.url

Or see: http://www.ncbi.nlm.nih.gov/pubmed/22973226

14-38

Lists of known pulmonary irritants in humans that have been evaluated in guinea pigs, rabbits, mice, etc. and cause a rapid shallow pattern and/or an increase in TP can be found in:

- Alarie, Y and Schaper, M (1988). Pulmonary performance in laboratory animals exposed to toxic agents and correlations with lung diseases in humans. In: Lung Biology in Health and Disease: Inhalation Toxicology. J Locke (Ed). Pp. 67-122, Marcel Dekker, Inc., NY.

14-39

- Alarie, Y, Iwasaki, M, and Schaper, M (1990). The use of whole body plethysmography in sedentary conditions or during exercise to determine pulmonary toxicity, including hypersensitivity, during or following exposure to airborne toxicants. J. Am. Coll. Toxicol. 9: 407-439.
- Alarie, Y, Nielsen, GD, and Schaper, M (2000). Animal bioassays for evaluation of indoor air quality. In: Indoor Air Quality Handbook. Spengler, JD, Samet, JM, and McCarthy, JF (Eds). McGraw-Hill, NY, pp. 23.1-23.49 (Chapter 23).

14-40

CHAPTER 15: PULMONARY SENSITIZERS

A. *DEFINITION*

Chemicals, which when inhaled, stimulate the immulogic system such that upon re-exposure, to even fairly low concentrations, a pulmonary reaction is induced.

Thus, we have two phases. The first phase is sensitization. It may result from a single exposure or from multiple exposures while the second phase is elicitation of the allergic reaction upon re-exposure. Note here that sensitization may occur by exposure routes other than inhalation.

15-1

B. *DIFFERENT TYPES OF REACTIONS*

➢ **TYPE I**	**Airways constriction with increased mucous secretion and inflammatory reaction of the airways (asthma)**
➢ **TYPE II**	**Cytotoxic response: hemolytic anemia**
➢ **TYPE III**	**Inflammatory reaction at the bronchioles and alveolar level (known as allergic alveolitis in England, and hypersensitivity pneumonitis in the U.S.)**
➢ **TYPE IV**	**Granuloma formation**

15-2

C. *DIFFERENT TIMES OF REACTIONS*

➢ **Immediate**	**within 30 minutes, Type I, asthma**
➢ **Late**	**4- 6 hours, Type I, asthma**
➢ **Delayed**	**8-24 hours: Type III, Hypersensitivity Pneumonitis**
➢ **Delayed**	**Type IV, granuloma formation**

D. *DIFFERENT MECHANISMS OF REACTIONS*

➢ **Type I**	**IgE antibody formation**
➢ **Type III**	**IgG antibody formation**
➢ **Type IV**	**Cell-mediated (T cells) (best example is beryllium)**

15-3

E. *DEFINITIONS*

<u>Asthma</u> (*see 15-6) is a lung disease with the following characteristics:

1. **Airway obstruction (or airway narrowing) that is reversible (but not completely in some patients) either spontaneously or with treatment.**
2. **Airway inflammation**
3. **Airway hyperresponsiveness to a variety of stimuli**

15-4

In all of its forms… "airway smooth muscle contraction which may result in airway obstruction is the primary abnormality in asthma". The typical symptoms (both intrinsic and extrinsic) are chest tightness, cough, wheezing, and dyspnea.

<u>Hypersensitivity Pneumonitis</u> (see 15-6) is a clinical disorder due to inhalation of particulates and is characterized in its acute phase by constitutional symptoms (e.g., fever, malaise, dyspnea), the presence of specific precipitating antibodies and by lymphocytic infiltration and sarcoid-type granulomas in the walls of alveoli and small airways. In its chronic phase, a progressive diffuse intrapulmonary fibrosis (DIPF) is observed.**

15-5

F. *AGENTS INVOLVED*

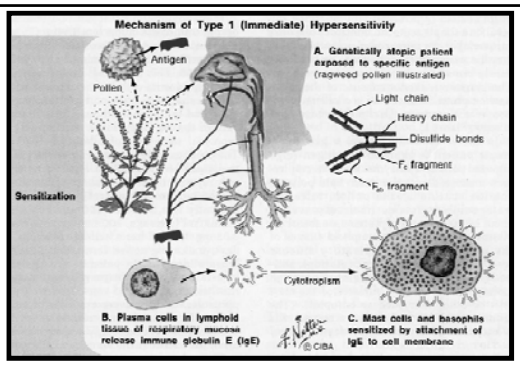
Environmental agents responsible are listed in Tables below. Very frequently exposure is from industrial processing and thus, the term "occupational asthma" is used*.**

***From: Guidelines for the Diagnosis of and Management of Asthma (1991). National Institutes of Health, Publication No. 91-3042-3042A:1.**
****From: Parkes, WR (1982). Occupational Lung Disorders, Butterworth, London.**

***** The following books can be consulted for more details:**
Bardana, EJ, Montanaro, A and O'Hollaren, MT (1992). Occupational Asthma. Hanley and Belfus, Inc.
Bernstein, IL, Chan-Yeung, M, Malo, J-L and Bernstein, DI (1999). Asthma in the workplace. Marcel Dekker, Inc.

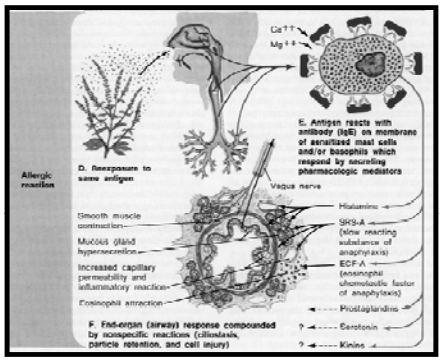
It should be noted here that asthma is becoming the most commonly compensated occupational lung disease.
See: Richman, SI (1993). Legal aspects of asthma in the workplace. Pennsylvania Bar Association Quarterly. Volume LXIV, No. 3, pages 161-171.

15-6



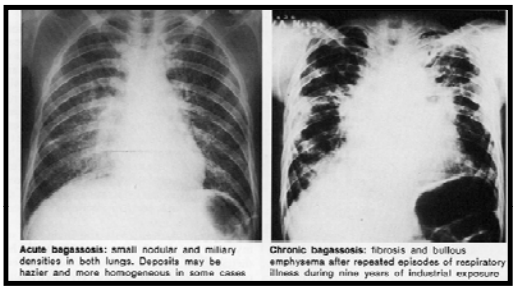
Salient features of allergic bronchial asthma, immediate Type I reaction. From Weiss, EB (1975). Bronchial Asthma, Ciba Clinical Symposia, Vol. 27. Reprinted with permission. Add to the above the presence (release) of the following cytokines: IL-3, IL-4, IL-5, IL,6, IL, 8 and others mediators, TNF-α, IFN-γ, etc.

15-7



As on 15-7: Salient features of allergic bronchial asthma, immediate Type I reaction. From Weiss, EB (1975). Bronchial Asthma, Ciba Clinical Symposia, Vol. 27. Reprinted with permission.

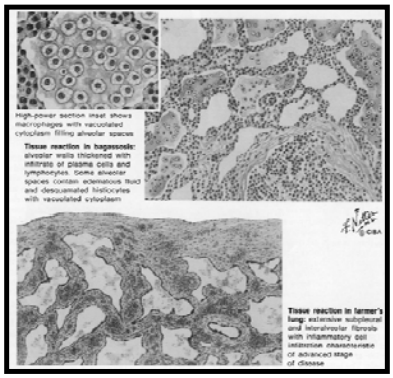
15-8



Salient features of acute and chronic hypersensitivity pneumonitis. From: Ziskind, MM (1978). Occupational Pulmonary Disease. Ciba Clinical Symposia, Vol. 30. Reprinted with permission.

This is a very good source for color illustrations of occupational pulmonary diseases caused by particulate inhalation such as asbestos, coal dust, beryllium, cadmium, silica, iron, etc.

15-9



As on 15-9: Salient features of acute and chronic hypersensitivity pneumonitis. From: Ziskind, MM (1978). Occupational Pulmonary Disease. Ciba Clinical Symposia, Vol. 30. Reprinted with permission.

15-10

TABLE 1. Causes of Occupational Asthma: Allergic Mechanism, High Molecular Weight Compounds[a]

Agents	Industries	Agents	Industries
Laboratory animals	Laboratory workers	**Biologic enzymes**	
Rats		B. Subtilis	Detergent industry
Mice	Veterinarians	Trypsin	Pharmaceutical
Rabbits		Papain	Laboratory
Guinea pigs	Animal handlers	Pancreatin	Pharmaceutical
		Pepsin	Pharmaceutical
		Bromelin	Pharmaceutical
		Fungal amylase	Manufacturing, bakers

[a]Abbreviated and modified from Chang-Yeung and Lam, S (1986). Occupational Asthma. Amer. Rev. Resp. Dis. 133, 686-703.

15-11

TABLE 1. Continued.

Agents	Industries	Agents	Industries
Birds		**Vegetables**	
Pigeons	Pigeon breeders	Gum acacia	Printers
Chickens	Poultry workers	Gum tragacanth	Gum manufacturing
Budgerigar	Bird fanciers	Peanuts	
Insects		**Other**	Crab processing
Grain mite	Grain workers	Crab	Prawn processing
Locust	Research workers	Prawn	Oyster farm
		Hoya	Sericulture
Cockroach	Research workers	Larva silk worm	
		Latex	Health workers

15-12

TABLE 1. Continued.

Agents	Industries
Plants	
Grain dust	Grain handlers
Wheat/rye flour	Bakers, millers
Buckwheat	Bakers
Coffee bean	Food processors
Castor bean	Castor oil industry
Tea	Tea workers
Tobacco leaf	Tobacco manufacturers
Hops	Brewery

Note: Some agents will induce an immediate reaction, others a late reaction, and some both an immediate and a late reaction. The mechanism is classified as allergic from skin tests, serum specific IgE, precipitin, and also confirmation of the effect can be obtained by a bronchoprovocation test (i.e., inhalation challenge by the suspected compound).

15-13

TABLE 2. Causes of Occupational Asthma: Allergic or Possibly Allergic Mechanism, Low Molecular Weight Compounds[a]

Agents	Industries	Agents	Industries
Diisocyanates		**Wood Dust**	
Toluene diisocyanate(TDI)	TDI production Polyurethane production	Western red cedar	Sawmill, Cabinet-making, Construction
		California red wood	
Diphenylmethane diisocyanate (MDI)	Foundries Electronic Polyurethane production	Many other woods	
Hexamethylene diisocyanate (HDI)	Automobile spray painting		

[a]Abbreviated and modified from Chang-Yeung and Lam, S (1986). Occupational Asthma. Amer. Rev. Resp. Dis. 133, 686-703.

15-14

TABLE 2. Continued.

Agents	Industries	Agents	Industries
Anhydrides		**Drugs**	
Phthalic anhydride	Epoxy resins, plastics	Penicillins	Pharmaceutical
		Cephalosporins	Pharmaceutical
Trimellitic anhydride	Epoxy resins, plastics	Piperazine	Pharmaceutical
		Psyllium	Pharmaceutical and nurses
Tetrachloro-phthalic anhydride	Epoxy resins, plastics		

15-15

TABLE 2. Continued.

Agents	Industries	Agents	Industries
Fluxes		**Other Chemicals**	
Aminoethyl Ethanolamine	Aluminum soldering	Dimethyl ethanolamine	
Colophony	Electronics industry	Persulfate salts	
		Henna	
Metals		Ethylene diamine	
Platinum		Hexachlorophene	
Nickel		Paraphenylene diamine	
Chromium			
Cobalt			
Vanadium			
Tungsten carbide			

15-16

TABLE 3. Agents Causing Type III Reaction, Hypersensitivity[a]

Type	Nature of Antigen
Farmer's Lung	*M. faeni, T. vulgaris, T. thalpophilus*
Bird Fancier's Lung	Avian proteins
Bagassosis	*T. sacchari*
Mushroom Worker's Lung	Thermoactinomecetes (e.g., *Actino-bifida dichotomica),* Mushroom spores
Malt Worker's Lung	*Aspergillus clavatus*
Suberosis	*Penicillum frequentans*
Air-Conditioner Disease (Humidifier Disease)	*T. vulgaris, T. thalpophilus,* Amaoebae: *N. gruberi and Acantamoeba*
Sauna-Taker's Disease	*Aureobasidium pullulans*
Sewage Sludge Disease	Gram negative bacteria
Diisocyanate Alveolitis	TDI and HDI

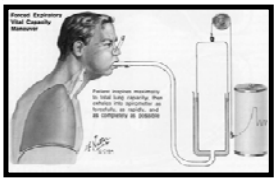
[a]Abbreviated from Parkes, WR (1982). Occupational Lung Disorders, Butterworth, London. Note that some agents listed here are also listed in Tables 1 and 2 for inducing a Type I reaction.

15-17

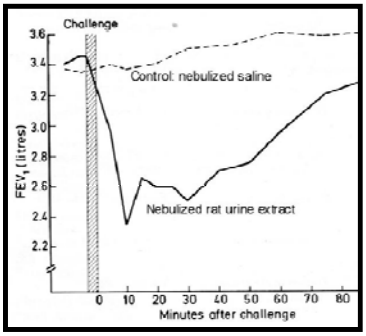
G. *BRONCHIAL PROVOCATION IN HUMANS*

Measurement of FEV1 is made before and at various time intervals following inhalation of the suspected agent.

Airways constriction, mucus secretion and inflammatory reaction of the conducting airways all yield a decrease in FEV1.



15-18



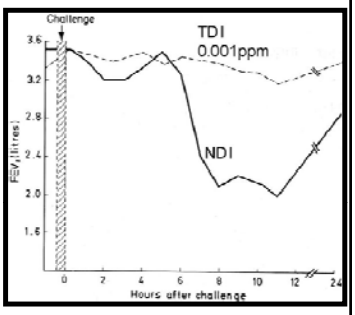
Note: Figure modified from Parkes.

This figure illustrates an immediate reaction following bronchial provocation with the suspected agent. The reduction in FEV1 is substantial.

Precautions are clearly needed when conducting such tests, such as having bronchodilators on hand, etc.

15-19

Note: Figure modified from Parkes.
This figure illustrates a late reaction. There is no reaction to TDI, while there is a strong reaction to NDI. Thus, one suspects that the reaction is not due to the irritating effect of NDI (all isocyanates are irritants), but that this individual has developed an allergic reaction to NDI. As seen on 15-19, The reduction in FEV1 is substantial.



Toluene diisocyanate (TDI)
Naphthalene diisocyanate (NDI)

15-20

H. ANIMAL MODELS OF TYPE I REACTION

1. Guinea pigs
Guinea pigs have been used for over 70 years. They can be easily sensitized by inhalation of ovalbumin (or other proteins) or chemicals such as TDI.

A typical protocol is to expose them 10-30 minutes/day for 5 consecutive days and wait 14-20 days before challenging them. Early investigators simply observed the animals for labored breathing due to bronchoconstriction during and after challenge. Increases in RL or decreases in EF50 have been used to monitor the response.

15-21

Widely used in the pharmaceutical industry to evaluate drugs capable or preventing or reversing bronchoconstriction.

However, the type of antibodies in guinea pigs is IgG1, not IgE as in humans. Furthermore, despite a dramatic bronchoconstriction, there is little inflammatory reaction as in humans and no or much less airway hyperresponsiveness.

15-22

2. Mice and rats
Mice have been used by immunologists and geneticists for over 30 years since sensitization elicits strong IgE production in some strains.

However upon challenge, they do not develop a strong bronchoconstriction. Thus, some physiological measurement is needed. A series of articles using BALB/c mice or the Brown Norway rat, with a sensitization protocol and measurements of EF50 during challenge is available at:

www.yvesalarie.com

as well as the article by Hoymann cited on 14-38.

15-23

The Brown Norway rat has a better developed bronchial musculature than BALB/c mouse and thus a stronger bronchoconstriction is elicited. Both develop an inflammatory reaction and airway hyperresponsiveness has been shown in both following challenges with an allergen. Furthermore, immediate and late reactions have been obtained and persistent airway inflammation can be induced with repeated challenges. Thus, there has been great progress in this field in the past few years and these models are closer to resembling asthma in human than the guinea pig model is.

15-24

Note: However, mice and rats, while reacting to cholinergic agonists, do not react to mediators of anaphylaxis, such as histamine and others, like the guinea pig does.

See: Martin, TR et al. (1988). Pulmonary responses to bronchoconstrictor agonists in the mouse. J. Appl. Physiol. 64: 2318-2323.

15-25

I. IRRITANT INDUCED ASTHMA

For many years, it has been suspected that a single exposure to a high concentration (accidental exposure) of some irritants (not all) can induce asthma, in the sense of a long lasting inflammatory reaction of the airways, leading to airways hyperresponsiveness. The condition was originally described as: Reactive Airways Dysfunction Syndrome (RADS)*.

*See: Brooks, SM and Bernstein, IL (1993). Reactive Airways Dysfunction Syndrome or Irritant-Induced Asthma. In: Asthma in the workplace. Bernstein, IL, Chan-Yeung, M, Malo, JL and Bernstein, DI (Eds). Marcel Dekker, NY, pp.533-549.

15-26

It may not be so easy to recognize it since allergic asthma or bacterial and viral infections all lead to airways hyperresponsiveness. However, using specific criteria and exposure history, it is clear that such can occur.

- **Documented absence of preceding respiratory complaints**
- **Onset of symptoms occurred after a single specific exposure**
- **Exposure involved a gas, smoke, fume, or vapor, which was present at a very high concentration and had irritant qualities**

15-27

- **Onset of symptoms occurred within 24 hours after exposure and persisted for at least three months**
- **Symptoms simulated asthma with cough, wheezing and dyspnea predominating**
- **Pulmonary function tests may show airflow obstruction**
- **Methacholine challenge was positive (i.e., lower methacholine than normal needed to decrease FEV1)**
- **Other types of pulmonary diseases ruled out**

15-28

TABLE 4. Chemicals or Mixtures Reported to Have Induced RADS

(Summarized and interpreted from original article by Brooks, Weiss and Bernstein)

Chemical	Summary of Exposure and Findings
Uranium hexafluoride gas	Exposure for 15 min during an accident. Emergency room, discharged 4 days later. Evaluated for RADS 140 months after exposure.*
Concrete floor sealant, containing the irritant epichloro-hydrin, along with a mixture of decane, ethyl-benzene, toluene and xylol	Exposed on Day 1 and Day 3 during application in a closed room. Developed dizziness, watery eyes, severe headaches, facial flushing, cough, and dyspnea. Examined a few days later by a physician: mild conjunctivitis, inflamed throat, runny eyes. Evaluated for RADS 14 months after exposure.

*Evaluation performed because of persistent respiratory symptoms in these patients.

15-29

TABLE 4. Continued

Chemical	Summary of Exposure and Findings
Vinyl latex primer, containing 25% ammonia, 16.6% aluminum chloro-hydrate and other additives	Spray painting for 12 hours in a closed room. Two painters developed generalized weakness, nausea, cough, shortness of breath, chest tightness, wheezing. Hospitalized two weeks, Evaluated for RADS 4 months after exposure.
Hydrazine solution exposure to face, mouth, neck, etc. and ingestion	Accidental splash of 35% hydrazine solution. Neck pain, respiratory symptoms, disoriented, hospitalized and treated with steroids. Evaluated 34 months after exposure.

15-30

TABLE 4. Continued

Chemical	Summary of Exposure and Findings
Spray paint	Spay painting operation in an enclosed area resulting in large amount of paint fumes of an oil-base enamel. Shortness of breath, wheezing, could no longer be around paint fumes. Evaluated for RADS 56 months after exposure.
Heated acid	Exposure while welding a tank which previously contained acid. Developed worsening cough, wheezing, shortness of breath. Evaluated for RADS 48 months after exposure.
Metal coat remover	Accidental inhalation of a large concentration of a coating-removing chemical believed to contain chlorine. Developed nausea, burning sensation on inspiration and paroxysmal coughing. Treated with corticosteroid and theophylline. Evaluated for RADS 39 months after exposure

15-31

TABLE 4. Continued

Chemical	Summary of Exposure and Findings
Fumigating fog, containing polyoxy-ethylated vegetable oil, dipropylene glycol, unsaturated aldehyde, isopropyl acetate, sodium nitrate, and turpine hydrocarbon	Exposure to thick brown fog. Developed gasping, choking sensation followed by wheezing, cough, tremendous rhinorrhea and treated with corticosteroids. Evaluated for RADS 6 months after exposure.
Fire smoke	Inhalation of smoke, fumes, combustion and pyrolysis products generated in a fire. Developed cough, shortness of breath, wheezing and chest discomfort. Evaluated for RADS 11 months after exposure.

15-32

Other chemicals suspected or demonstrated to have caused RADS, after exposure to a HIGH concentration:

- **SO_2**
- **Ammonia**
- **Fumes from drain cleaning agents**
- **Chlorine**
- **Toluene Diisocyanate**
- **Ozone**
- **Methyl isocyanate**
- **Smoke from burning polyvinyl chloride**

15-33

CHAPTER 16: CHRONIC EFFECTS

A. *CHRONIC BRONCHITIS*

Definition: Condition associated with hyperplasia (more numerous) and hypertrophy (larger size) mucus secreting glands found in the submucosa of the large airways. Also, there is hyperplasia of goblet cells in the smaller airways with smooth muscle hypertrophy and peribronchial fibrosis.

It is characterized by chronic cough and sputum production, but the net result is chronic obstructive lung disease with hyperinflated lung, loss of pulmonary reserve capacity, and great difficulty in getting air out of the lung. 16-1

It is caused by infections and chronic exposure to irritants. The best example being cigarette smoking. Net result is chronic obstructive pulmonary disease (COPD).

B. *EMPHYSEMA*

Definition: Condition associated with septal destruction and dilation within terminal respiratory units (i.e., alveolar walls as well as pulmonary capillaries destruction resulting in hyperinflated lungs). The net result is also COPD. Caused by α-1 antitrypsin deficiency and cadmium fumes.

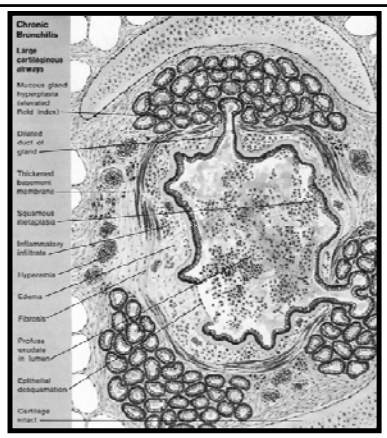
16-2

C. *PNEUMOCONIOSIS*

Definition: A non-neoplastic reaction of the lungs to inhaled mineral or organic *dust* and the resultant alteration in their structure, excluding asthma, bronchitis and emphysema. In several cases (e.g., silicosis, asbestosis), the lung becomes stiffer because of fibrosis and the net result is called "restrictive lung disease" because the patient has difficulties taking a deep breath, but unlike COPD there is no airflow obstruction during exhalation.

D. *INHALED CHEMICALS CAPABLE OF INDUCING CANCERS* (see next chapter)

16-3



16-4

Classification of pneumoconiosis, with some examples[a]

Type of Dust	Lung Reaction	Examples
↑	No fibrosis: "inert dust" Local macrophage accumulation; little structural change; mild reticulin proliferation	Soot Iron oxide (siderosis) Tin (stannosis) Barium (baritosis)
Mineral	Color changes of lung tissues (brick red with iron, blue black with coal)	Early stages of coal pneumoconiosis
↓	Sarcoid-type granulomas Foreign body granulomas	Beryllium disease Talc

[a]From: Parkes, as above (see 15-17)

16-5

Classification of pneumoconiosis, with some examples[a] (continued)

Type of Dust	Lung Reaction	Examples
↑	nodular or massive	Quartz and certain other forms of free silica (silicosis) "Mixed Dust" fibrosis Later stages of coal pneumoconiosis
Mineral	Collagenous fibrosis	_____
↓	diffuse interstitial	Asbestos (asbestosis) "Talc" pneumoconiosis Beryllium disease Hard metal disease

[a]From: Parkes, as above (see 15-17)

16-6

Classification of pneumoconiosis, with some examples[a] (continued)

Type of Dust	Lung Reaction	Examples
Non-mineral (organic) e.g., actinomycete spores, avian and animal proteins	No fibrosis in acute phase Transient "interstitial pneumonia" or sarcoid-type granuloma formation (acute extrinsic allergic "alveolitis") Collagenous fibrosis (chronic extrinsic allergic alveolitis -diffuse interstitial pulmonary fibrosis (DIPF)	Farmers' Lung Mushroom Workers' Lung Bagassosis Bird Fanciers' Lung Farmer's Lung Bagassosis Bird Fancier's Lung

[a]From: Parkes, as above (see 15-17)

Note: See Chapter 18 for a recent occupational pulmonary disease

16-7

CHAPTER 17:
STANDARDS FOR EXPOSURE TO AIRBORNE CONTAMINANTS

A. *STANDARDS OR GUIDELINES*

A variety of standards have been promulgated. Some are Federal standards, while others are guidelines promulgated by different groups.

Table 1 on 17-8 to 17-12 provides a summary and the definition of the most important standards.

17-1

1. National Ambient Air Quality Standards (NAAQS)

These are established by the US EPA, following a review of the literature on a particular substance. The review is published as an "Air Quality Criteria Document" and is readily available in libraries. The current NAAQS are listed in a table below.

2. Threshold Limit Values (TLV) and Biological Exposure Indices (BEI)

These are established by the American Conference of Governmental Industrial Hygienists (ACGIH) which was established in 1938.

17-2

The ACGIH started issuing exposure guidelines in 1946 for a small number of industrial chemicals and there is currently a TLV for about 600 industrial chemicals. Each year, it published a booklet listing those values.

This booklet is available from:

ACGIH
1330 Kemper Meadow Drive
Cincinnati, OH 45240-1634
http://www.acgih.org

A page taken from this booklet has been reproduced below.

17-3

More recently, the ACGIH has introduced Biological Exposure Indices (BEIs) as reference values, intended as guidelines for evaluation of potential health hazards in the practice of industrial hygiene. These, unlike TLVs which refer to an air concentration, are for an amount of the chemical or its metabolite/s in blood, urine, exhaled air, etc. There are currently 39 established BEIs.

Both the TLVs and BEIs are established after a literature review, much more extensive for BEIs than for TLVs and this review is published in "The Documentation of the Threshold Limit Values and Biological Exposure Indices".

17-4

In this publication, you can find the *basis* for establishing a TLV or BEI. Many TLVs have been recently reviewed and much more extensive documentation is now available.

Also, there have been obvious changes over the years as to what the committee members regard as an "adverse health effect" and what "most workers" means. Twenty years ago, "most workers" was about 85%. Now I don't know.

It should be kept in mind that these values were guidelines, developed initially for Normal, Healthy, Adult, Male, not female and certainly not pregnant females.

17-5

Before you can use these values, you should read the "Preface" which is now 10 pages long in the booklet. Also, you should read the accompanying Documentation for each substance in which you are interested.

3. Permissible Exposure Limit (PEL)

These are established by the US Occupational Safety and Health Administration (OSHA). They are the law. The 1970 Act adopted the 1968 TLVs initially as PEL.

17-6

4. Others

The National Institute of Occupational Safety and Health (NIOSH) develops Recommended Exposure Levels (REL) and transmits such to OSHA for use in promulgating legal standards (i.e., PEL). Also, several states are active in promulgating "Air Toxics" standards.

17-7

Table 1. Standards, Definitions, and Promulgating Agencies for Airborne Contaminants[a]

Standard	Responsible Agency or Group	Definition
Threshold Limit Value (TLV) Time-Weighted Average (TWA)	American Conference of Governmental Industrial Hygienists (ACGIH)	The time-weighted average concentration for a normal 8-hour workday or 40-hour workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.
Threshold Limit Value – Ceiling (TLV-C)	ACGIH (as above)	The concentration that should not be exceeded even instantaneously.

[a]From: Kane, LE et al. (1979). Amer. Ind. Hyg. J. 40: 207-229.

17-8

Table 1. Continued

Standard	Responsible Agency or Group	Definition
Short-Term Exposure Limit (STEL)	ACGIH (as above)	The maximal concentration to which workers can be exposed for a period of up to 15 minutes continuously without suffering from 1) intolerable irritation, 2) chronic or irreversible tissue change, or 3) narcosis ... provided that no more than four excursions per day are permitted ... the STEL should be considered a maximal allowable concentration, or absolute ceiling, not to be exceeded ...

17-9

Table 1. Continued

Standard	Responsible Agency or Group	Definition
Permissible Exposure Limit (PEL)[b]	Occupational Safety and Health Administration (OSHA)	Set in accordance with Sec 6(b)5 of Public Law 91 596....standard which most adequately assures to the extent feasible, on the basis of the best available evidence, that no employee will suffer material impairment of health or functional capacity even if such employee has regular exposure to the hazard dealt with by such standard for the period of his working life.

[b]See note on 17-12.

17-10

Table 1. Continued

Standard	Responsible Agency or Group	Definition
Emergency Exposure Limit (EEL)	Committee on Toxicology, National Academy of Sciences	The EEL for short-term exposure to an airborne contaminant is a concentration which, when inhaled for a specified single, brief period, rare in the lifetime of an individual, is believed not to result in a period of disability for interference with the performance of his assigned task.

17-11

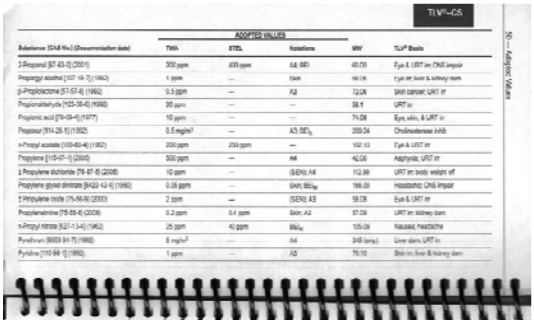
Table 1. Continued

Standard	Responsible Agency or Group	Definition
National Ambient Air Quality Standard (NAAQS)	Environmental Protection Agency	They prescribe pollutant exposures or levels of effect that a political jurisdiction determines should not be exceeded in a specific geographic area …

[b]Note: The 1968 TLVs (as 8-hour TWAs) have been designated as consensus standards (i.e., PELs) by OSHA in 1970, until a new PEL is established independently by OSHA. This was changed in 1989, when the 1987-1988 TLVs (as 8-hour TWAs) were designated consensus standards by OSHA. However, this change was challenged in Federal Court, and reversed. Because of this reversal, the most followed "occupational exposure limits" in industry in the US today are those established by the ACGIH, unless the PEL established independently by OSHA is lower than the TLV established by the ACGIH. This is an unfortunate situation and has created much confusion.

17-12

Copy of a page from the 2013 TLVs and BEIs booklet of the ACGIH.



17-13

Each substance is listed with the TLV, as an 8-hr time weighted average (TWA), then the STEL (if any) and appropriate notations such as 'skin'. Chemicals may have any effect on the skin or be absorbed via the skin. The last column gives the basis for the TLV (i.e., the critical health effect/s that the chemical substance may have above the TLV, or the toxic effect/s for which the TLV has been set. See pages 73-74 of the 2013 booklet for additional information.

From page 73:
A complete list of the TLV bases used by the Threshold Limit Values for Chemical Substances Committee may be found in their Operations Manual online at:
http://www.acgih.org/TLV/Approved_Revised_TLV-CS_Comm_Ops_Manual-final.pdf

17-14

OSHA also adopted a similar system of classifying health effects for each substance for which a PEL is established. I did an analysis of the health effects cited in the 1999 TLV booklet. A total of 65 different health effects were cited as shown in the table on page 17-17. After coding each effect, a statistical analysis was conducted to find the most frequently cited effects. The results are shown in the figure on page 17-18. After coding, it is also easy to sort the table by cited effects, list the chemicals within cited effects, and compare how the TLV was arrived at within each group. An interesting exercise.

17-15

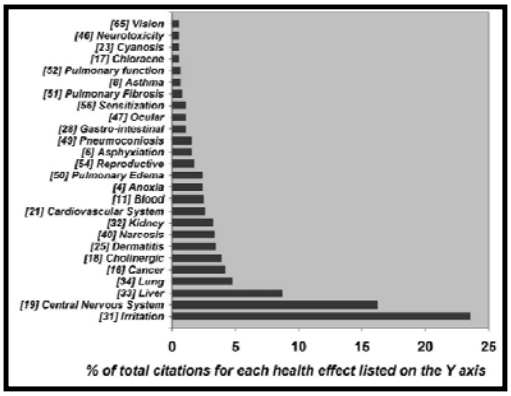
On page 17-17:
Table of "health effects" or "toxic effects" cited in TLV booklet of 1999, listed alphabetically. Each effect was coded with a number that can be seen in front of each effect in the table below. A statistical analysis was then conducted to find the types of effects cited most often.

On page 17-18:
The results are seen in the figure, for the most frequently cited effects.

17-16

1 Anemia	23 Cyanosis	45 Neuropathy
2 Anesthesia	24 Depigmentation	46 Neurotoxicity
3 Anosmia	25 Dermatitis	47 Ocular
4 Anoxia	26 Fibrosis	48 Ototoxic
5 Argyria	27 Fluorosis	49 Pneumoconiosis
6 Asphyxiation	28 Gastro-intestinal	50 Pulmonary Edema
7 Asbestosis	29 Headache	51 Pulmonary Fibrosis
8 Asthma	30 Immunotoxicity	52 Pulmonary Function
9 Berylliosis	31 Irritation	53 Recurrent Fever
10 Bleeding	32 Kidney	54 Reproductive
11 Blood	33 Liver	55 Seizures
12 Bone	34 Lung	56 Sensitization
13 Bronchitis	35 Metabolic Disorders	57 Siderosis
14 Burns	36 Metal Fume Fever	58 Silicosis
15 Byssinosis	37 Mucous Membrane	59 Skin
16 Cancer	38 Mucostasis	60 Stannosis
17 Chloracne	39 Muscle Toxin	61 Sudden Death
18 Cholinergic	40 Narcosis	62 Teeth
19 Central Nervous System	41 Nasal	63 Thyroid
20 Corrosion	42 Nausea	64 Urticaria
21 Cardiovascular System	43 Necrosis	65 Vision
22 Coal Worker Pneumoconiosis	44 Neoplasia	

17-17



17-18

Suspected or Confirmed Human Carcinogens in the 2013 TLV Booklet

Type[a]	Substance	Adopted Values (TWA) ppm	mg/m³
A1	4-Aminodiphenyl	-	-
A2	Antimony trioxide, production	-	-
A1	Arsenic and inorganic compounds, as As		0.01
A1	Asbestos, all forms	-	0.1 fiber/cc
A1	Benzene	0.5	-
A1	Benzidine	-	-

[a]See end-notes on 17-26

17-19

Type	Substance	Adopted Values (TWA) ppm	mg/m³
A2	Benzo(b)fluoroanthene	-	-
A2	Benzo(a)pyrene	-	-
A2	Benzotrichloride	C0.1	-
A1	Beryllium and compounds, as Be	-	0.00005
A2	1,3-Butadiene	2.0	-
A2	Cadmium and compounds, as Cd	-	0.01 0.002 (*R)
A2	Cadmium chromate	-	0.001

*Note: R designates respirable fraction

17-20

Type	Substance	Adopted Values (TWA) ppm	mg/m³
A2	Carbon tetrachloride	5.0 STEL10	-
A1	bis (Chloromethyl ether)*	0.001	
A2	Chloromethyl methyl ether	-	-
A1	Chromium, and inorganic compounds, as Cr Water-soluble Cr VI compounds Insoluble Cr VI compounds	 - -	 0.05 0.01
A1	Coal tar pitch volatiles, as benzene soluble aerosol	-	-

*Note: Probably the most potent and fast-acting carcinogen in humans. It induced bronchocarcinoma in young adult male workers.

17-21

Type	Substance	Adopted Values (TWA) ppm	mg/m³
A2	Diazomethane	0.2	-
A2	1,4-Dichloro-2-butene	0.005	-
A2	Dimethyl carbamoyl chloride	0.005	-
A2	Ethylene oxide	1.0	-
A2	Formaldehyde	C0.3	-
A2	Lead chromate, as Pb , as Cr	- -	0.05 0.012
A2	MBOCA: 4,4'-Methylene bis(2-chloroaniline)	0.01	-

17-22

Type	Substance	Adopted Values (TWA) ppm	mg/m³
A2	β-Naphthylamine	-	-
A1	Nickel and inorganic compounds, as Ni Insoluble inorganic compounds Nickel subsulfide, as Ni	 - -	 0.2 0.1
A2	4-Nitrodiphenyl	-	-
A1	Silica, crystalline – α-quartz and cristobolite	-	0.025
A2	Silicon carbide, fibrous (including whiskers)	-	0.1 f/cc

17-23

Type	Substance	Adopted Values (TWA) ppm	mg/m³
A2	Strontium chromate, as Cr	-	0.0005
A2	Sulfuric acid	-	0.2
A2	Synthetic vitreous fibers Refractory ceramic fibers	-	0.2 f/cc
A1	Talc Containing asbestos fibers	-	*See asbestos
A2	Trichloroethylene	10 STEL 25	-
A1	Uranium Soluble and insoluble compounds, as U	-	0.2 STEL 0.5

17-24

Type	Substance	Adopted Values (TWA) ppm	mg/m³
A2	Vinyl bromide	0.5	-
A1	Vinyl chloride	1.0	-
A2	Vinyl fluoride	1.0	-
A1 A2	Wood dust Oak and Beech Birch, Mahogany, Teak, and Walnut	 - -	 - -
A1	Zinc chromates, as Cr	-	0.01

Note: Not listed above is another very potent human respiratory tract carcinogen, sulfur mustard or mustard gas. Discovered in Japanese workers after WW II.

17-25

[a] A1 or A2 listed substances. Important to remember are the A1 chemicals. Although these chemicals induce tumors mostly in the respiratory tract when inhaled, some do not. Vinyl chloride, for example, induces liver cancer (angiosarcoma), while β-naphthylamine induces bladder cancer.

Categories of inhaled chemicals as carcinogens from the ACGIH.

A1 Confirmed human carcinogen
A2 Suspected human carcinogen
A3 Confirmed animal carcinogen, with unknown relevance to humans
A4 Not classifiable as a human carcinogen
A5 Not suspected as a human carcinogen

17-26

National Ambient Air Quality Standards (NAAQS)

The Clean Air Act, which was last amended in 1990, requires EPA to set National Ambient Air Quality Standards (40 CFR part 50) for pollutants considered harmful to public health and the environment. The Clean Air Act identifies two types of national ambient air quality standards.

http://www2.epa.gov/laws-regulations/summary-clean-air-act

http://www.epa.gov/airscience/air-airqualitystandards.htm

17-27

Primary standards provide public health protection, including protecting the health of "sensitive" populations such as asthmatics, children, and the elderly. ***Secondary standards*** provide public welfare protection, including protection against decreased visibility and damage to animals, crops, vegetation, and buildings.

EPA has set National Ambient Air Quality Standards for six principal pollutants, which are called "criteria" pollutants. They are listed on 17-29, 17-30, and 17-31. Units of measure for the standards are parts per million (ppm) by volume, parts per billion (ppb) by volume, and micrograms per cubic meter of air (μg/m^3).

17-28

Pollutant [final rule cite]	Primary/ Secondary	Averaging Time	Level	Form
Carbon Monoxide (CO) [76 FR 54294, 08-31-11]	Primary	8-hr 1-hr	9 ppm 35 ppm	Not to be exceeded > 1x per yr
Lead (Pb) [73 FR 66964, 11-12-08]	Primary & Secondary	Rolling 3-month average	[a]0.15 μg/m^3	Not to be exceeded
Nitrogen Dioxide (NO_2) [75 FR 6474, 02-09-10] [61 FR 52852, 10-08-96]	Primary Primary & Secondary	1-hr Annual	100 ppb [b]53 ppb	98th percentile, averaged over 3 yrs Annual mean

[a,b] See 17-32

17-29

Pollutant [final rule cite]	Primary/ Secondary	Averag-ing Time	Level	Form
Ozone [73 FR 16436, 03-27-08]	Primary & Secondary	8-hr	[c]0.075 ppm	Annual 4th highest daily maximum 8-hr concentration, averaged over 3 yrs
Particle pollution PM2.5	Primary Secondary Primary & Secondary	Annual Annual 24-hr	12 μg/m^3 15 μg/m^3 35 μg/m^3	Annual mean, averaged over 3 yrs 98th percentile, averaged over 3yrs

[c] See 17-32 and 17-33

17-30

Pollutant [final rule cite]	Primary/ Secondary	Averag-ing Time	Level	Form
Particle pollution PM10 [12-14-12]	Primary & Secondary	24-hr	150 μg/m^3	Not to be exceeded > 1x per yr, on average over 3 yrs
Sulfur dioxide (SO_2) [75 FR 35520, 06-22-10] [38 FR 25678, 09-14-73]	Primary Secondary	1-hr 3-hr	[d]75 ppb 0.5 ppm	99th percentile of 1-hour daily maximum concentrations, averaged over 3 years Not to be exceeded > 1x per yr

[d] See 17-33

17-31

As of October 2011

a Final rule signed October 15, 2008. The 1978 lead standard (1.5 μg/m^3 as a quarterly average) remains in effect until one year after an area is designated for the 2008 standard, except that in areas designated nonattainment for the 1978, the 1978 standard remains in effect until implementation plans to attain or maintain the 2008 standard are approved.

b The official level of the annual NO_2 standard is 0.053 ppm, equal to 53 ppb, which is shown here for the purpose of clearer comparison to the 1-hr standard.

c Final rule signed March 12, 2008. The 1997 ozone standard (0.08 ppm, annual 4th highest daily maximum 8-hr concentration, averaged over 3 yrs) and related implementation rules remain in place.

17-32

c Continued from 18-6 …
In 1997, EPA revoked the 1-hr ozone standard (0.12 ppm, not to be exceeded > 1x per yr) in all areas, although some areas have continued obligations under that standard ("anti-backsliding"). The 1-hr ozone standard is attained when the expected number of days per calendar year with maximum hourly average concentrations above 0.12 ppm is ≤ 1.

d Final rule signed June 2, 2010. The 1971 annual and 24 hour SO_2 standards were revoked in that same rulemaking. However, these standards remain in effect until 1 yr after an area is designated for the 2010 standard, except in areas designated nonattainment for the 1971 standards, where the 1971 standards remain in effect until implementation plans to attain or maintain the 2010 standard are approved.

17-33

See the historical tables of NAAQS standards

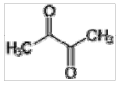
- **Carbon Monoxide**
http://www.epa.gov/ttn/naaqs/standards/co/s_co_history.html
- **Lead**
http://www.epa.gov/ttn/naaqs/standards/pb/s_pb_history.html
- **Nitrogen Dioxide**
http://www.epa.gov/ttn/naaqs/standards/nox/s_nox_history.html
- **Ozone**
http://www.epa.gov/ttn/naaqs/standards/ozone/s_o3_history.html
- **Particle Pollution**
http://www.epa.gov/ttn/naaqs/standards/pm/s_pm_history.html
- **Sulfur Dioxide**
http://www.epa.gov/ttn/naaqs/standards/so2/s_so2_history.html

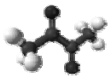
17-34

CHAPTER 18: RECENT OCCUPATIONAL PULMONARY DISEASE

DIACETYL (2,3-Butanedione) (CH_3-CO-CO-CH_3)

This chemical is a widely used flavoring agent and was widely used in the manufacturing of microwave popcorn. It is naturally occurring in some foods and beverages and seems to have a low toxicity when taken orally.





18-1

However, when inhaled, such as during manufacturing or used as artificial butter flavoring (with some elevated processing temperature favoring vapor release), this chemical can induce a condition known as bronchiolitis obliterans (BO) in exposed workers. This is a severe obstructive lung disease with inflammation and fibrosis (scar tissue) of the bronchioles and is irreversible.

Many possible causes for BO have been presented. Diacetyl was implicated as a possible cause starting around the year 2000 and various terms such as "Popcorn Lung", "Popcorn Workers Lung", etc. were used since it started to be observed in workers at a particular microwave popcorn factory in Missouri.

18-2

Toxicologists tried to induce BO with exposures of mice or rats to vapors of diacetyl, but this chemical is highly retained in the upper portions of the respiratory tract of these rodents and while severe inflammatory reactions and necrosis were observed in these upper portions, they were not observed at the lower conducting airways and no BO was observed. Interestingly, it was found to be a weak sensory irritant, with no activity as a pulmonary irritant, but it did induce airflow limitation in mice.

Since it is only a weak sensory irritant there is little warning to workers of possible toxic exposure. Its pleasant odor may even provide some reassurance, although this is speculative.

18-3

In 2011, Palmer et al. (see article below) reported that BO could be induced in rats with a single intratracheal instillation of diacetyl as examined at necropsy shortly (days) after treatment. Their extensive investigation leaves no doubt about the capability of diacetyl to induce BO. This article also provides a review of prior findings in animals and humans regarding diacetyl exposures.

In 2012, the ACGIH established a TLV of 0.01 ppm and a STEL of 0.02 ppm for diacetyl. OSHA has not yet established a PEL for this chemical, but industry will follow the ACGIH recommendations and a variety of changes have been made in the handling and use of diacetyl.

18-4

Severe Airway Epithelial Injury, Aberrant Repair and Bronchiolitis Obliterans Develops After Diacetyl Instillation in Rats

Scott M. Palmer[1]*, Gordon P. Flake[2], Fran L. Kelly[1], Helen L. Zhang[1], Julia L. Nugent[1], Patrick J. Kirby[2], Julie F. Foley[2], William M. Gwinn[2] and Dan L. Morgan[2].

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18-5

BACKGROUND:

Bronchiolitis obliterans (BO) is a fibrotic lung disease that occurs in a variety of clinical settings, including toxin exposures, autoimmunity and lung or bone marrow transplant. Despite its increasing clinical importance, little is known regarding the underlying disease mechanisms due to a lack of adequate small animal BO models. Recent epidemiological studies have implicated exposure to diacetyl (DA), a volatile component of artificial butter flavoring, as a cause of BO in otherwise healthy factory workers. Our overall hypothesis is that DA induces severe epithelial injury and aberrant repair that leads to the development of BO. Therefore, the objectives of this study were 1) to determine if DA, delivered by intratracheal instillation (ITI), would lead to the development of BO in rats and 2) to characterize epithelial regeneration and matrix repair after ITI of DA.

18-6

METHODS AND MAIN RESULTS:

Male Sprague-Dawley rats were treated with a single dose of DA (125 mg/kg) or sterile water (vehicle control) by ITI. Instilled DA resulted in airway specific injury, followed by rapid epithelial regeneration, and extensive intraluminal airway fibrosis characteristic of BO. Increased airway resistance and lung fluid neutrophilia occurred with the development of BO, similar to human disease. Despite rapid epithelial regeneration after DA treatment, expression of the normal phenotypic markers, Clara cell secretory protein and acetylated tubulin, were diminished. In contrast, expression of the matrix component Tenascin C was significantly increased, particularly evident within the BO lesions.

18-7

CONCLUSIONS:

We have established that ITI of DA results in BO, creating a novel chemical-induced animal model that replicates histological, biological and physiological features of the human disease. Furthermore, we demonstrate that dysregulated epithelial repair and excessive matrix Tenacin C deposition occur in BO, providing new insights into potential disease mechanisms and therapeutic targets.

For this article, see:
PLoS One. 2011; 6(3): e17644.
Published online 2011 March 25.
doi: 10.1371/journal.pone.0017644

18-8

An excellent review on diacetyl is available with a very insightful discussion on the recognition of occupational pulmonary diseases.

Kreiss, K (2012). Respiratory disease among flavoring-exposed workers in food and flavoring manufacture. Clin. Pulm. Med. 19: 165-173.

Also at:
http://journals.lww.com/clinpulm/Abstract/2012/07000/Respiratory_Disease_Among_Flavoring_exposed.3.aspx

18-9

ABSTRACT

Fixed airways obstruction was found in workers producing microwave popcorn in relation to inhaling synthetic butter flavoring volatiles in 2000. Since then, an industry-wide hazard of clinical bronchiolitis obliterans was found in other microwave-popcorn plants, in flavoring manufacture, and in diacetyl (2,3-butanedione) manufacture. Recently, workers in 1 food production and 1 flavoring manufacturing facility have had excesses of spirometric restrictive abnormalities. Evidence of flavoring-related excessive declines in forced expiratory volume in 1 second (FEV_1) suggests that restriction in the latter flavoring plant is work-related. However, the pathologic and physiological correlates of restriction in flavoring-exposed workers remain uninvestigated. Diacetyl vapor causes respiratory epithelial necrosis in rodents, compatible with the pathologic mechanism for constrictive bronchiolitis, but exposures in flavoring manufacturing are more diverse than diacetyl.

18-10

ABSTRACT (continued from 18-10)

The diacetyl substitute, 2,3-pentanedione, has comparable toxicity to diacetyl, and other members of the α-diketone family have not been evaluated for respiratory toxicity. With the increasing spectrum of flavoring-related lung diseases and chemical exposures, pulmonologists caring for flavoring-exposed workers have novel challenges. These include examining excessive FEV_1 declines in serial spirometry and improving surveillance spirometry quality so that excessive declines can be detected at an earlier stage. The best means of preventing permanent impairment from irreversible occupational lung disease is to intervene for workers with excessive FEV_1 decline within the normal range and before diagnostic criteria for occupational lung disease can be met. Regulation of diacetyl and 2,3-pentanedione, which does not yet exist, may not prevent all occupational lung disease in flavoring-exposed workers.

18-11

CHAPTER 19: UPTAKE OF CARBON MONOXIDE

Carbon monoxide (CO) has no direct effect on the respiratory tract and is odorless. Therefore there is no warning of exposure. Following absorption in blood, it will react with hemoglobin to form carboxyhemoglobin (COHb), thus reducing the amount of oxygen that can be carried to the tissues. Asphyxiation with this gas has been a very common problem and it is important to understand how it is absorbed in blood and possibly reach a lethal level (>50% COHb).

19-1

The basic considerations were described by Coburn, RF, Forster, RE and Kane, PB (1965). Considerations of the physiological variables that determine the blood carboxyhemoglobin concentration in man. J. Clin. Invest. 44: 1910.

They provided an equation (the CFK equation) to calculate COHb at any time during exposure to CO concentrations, as well as to calculate the release of CO from blood after exposure.

19-2

The most important basic variables and values for humans are:

1. VA: alveolar ventilation = 6,000 mL/min at rest, 18,000 at light work, and 30,000 at heavy work

2. DL: diffusing capacity for CO from alveolar air to capillary blood = 30mL/min/mmHg, 40 at light work and 60 at heavy work. Thus, rapidity of CO uptake is dependent (or limited) on both VA and DL.

3. Vb: volume of blood to be saturated= 5500 mL

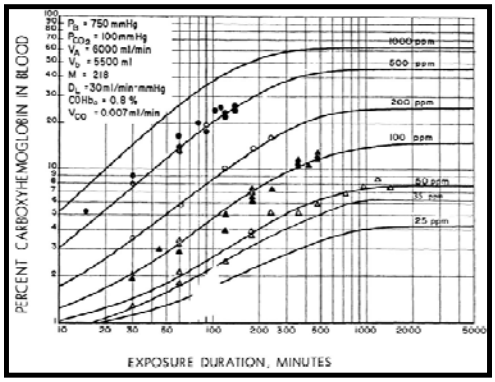
19-3

4. M: ratio of affinity of blood (hemoglobin) for CO to O2 is usually taken as 218

5. PICO: partial pressure of CO in mmHg (convert ppm concentration to mmHg) in inspired air is the driving force. The higher the concentration, the faster the uptake will be and obviously higher COHb levels will be reached.

6. t: duration of exposure. COHb will increase with exposure time until partial pressure equilibrium with inspired air (or death) is reached.

19-4



Uptake to equilibrium is illustrated above. This figure is from: Stewart, RD et al. (1970). Arch. Environ. Health 21: 154-164.

19-5

On 19-5, The solid lines are predicted from the CFK equation while the plotted data points are from measurements from exposed human volunteers. Not shown is the elimination. The half-life is about 4 hours and much shorter if pure O_2 is given to a victim. In fire situations, 20,000 to 40,000 ppm CO can be easily reached and thus 50% and much higher (up to 85%) COHb can be reached within a few minutes. At such high concentrations, the time difference between humans and a canary or a mouse reaching 50% COHb is much too small to be of importance. Instead, please purchase a smoke alarm!

The current ACGIH TLV for 8 hours is 25 ppm, the current OSHA PEL for 8 hours is 50 ppm and the EPA NAAQS for 8 hours exposure is 9 ppm and 35 ppm for 1 hour. No significant toxicological effects are expected below 5% COHb.

19-6