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阅读有关洗衣粉毒性及联合毒性作用评价方面的材料.

第三章　环境化学物的毒性作用及其影响因素

By Rutao Liu

§3-1毒性作用

一、基本概念

(一)毒物/toxicant

What is Toxicant?

指在一定条件下，较小剂量就能引起机体功能性或器质性损伤的化学物质。

Is there exist toxicant or non-toxicant?

毒物和非毒物没有绝对的界限，只能以中毒剂量的大小相对地加以区别。

(二)毒性/toxicity. What is toxicity?

毒性是一种物质对机体造成损害的性质和能力。毒性较高的物质，只要相对较小的数量，则可对机体、造成一定的损害；而毒性较低的物质，需要较多的数量，才呈现毒性。物质毒性的高低仅具有相对意义。在一定意义上，只要达到一定数量，任何物质对机体都具有毒性；在一般情况下，如果低于一定数量，任何物质都无毒性；关键是此种物质与机体接触的量。除物质与机体接触的数量外，还与物质本身的理化性质以及其与机体接触的途径有关。

化学物质毒性数据库

(三) 中毒/toxication: How can you get into toxication?

- 中毒是指机体受到某种化学物质的作用而产生功能性或器质性的病变。根据中毒发生、发展的快慢，可分为急性中毒、亚慢性中毒和慢性中毒。

*(四) 危险度与危害性

What is risk?

危险度：指在一定暴露条件下化学物导致机体产生某种不良效应的概率。

危险度分类：

1.归因危险度：

2.相对危险度：

3.可接受危险度：

危害性：一般化学污染物对机体可能产生危害的可能性。

(五)剂量/dose

剂量是决定外来化合物对机体损害作用的重要因素。

指给予机体的外来化合物数量或机体接触的数量。剂量的单位是以每单位体重接触的外来化合物数量表示，例如**mg/kg**体重。

1、致死量/Lethal amount

⑴、绝对致死量(LD100):

⑵、半数致死量(Lethal Dose, 50%：LD50):

表示LD50的单位mg/kg体重，LD50数值越小，表示外来化合物毒性越强；反之，LD50数值越大，则毒性越低。单位：mg/Kg

What does LD50 mean?

- LD stands for "Lethal Dose". LD50 is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals. The LD50 is one way to measure the short-term poisoning potential (acute toxicity) of a material.
- Toxicologists can use many kinds of animals but most often testing is done with rats and mice. It is usually expressed as the amount of chemical administered (e.g., milligrams) per 100 grams (for smaller animals) or per kilogram (for bigger test subjects) of the body weight of the test animal. The LD50 can be found for any route of entry or administration but dermal (applied to the skin) and oral (given by mouth) administration methods are the most common.

Why study LD50's?

- Chemicals can have a wide range of effects on our health. Depending on how the chemical will be used, many kinds of toxicity tests may be required.
- Since different chemicals cause different toxic effects, comparing the toxicity of one with another is hard. We could measure the amount of a chemical that causes kidney damage, for example, but not all chemicals will damage the kidney. We could say that nerve damage is observed when 10 grams of chemical A is administered, and kidney damage is observed when 10 grams of chemical B is administered. However, this information does not tell us if A or B is more toxic because we do not know which damage is more critical or harmful.
- Therefore, to compare the toxic potency or intensity of different chemicals, researchers must measure the same effect. One way is to carry out lethality testing (the LD50 tests) by measuring how much of a chemical is required to cause death. This type of test is also referred to as a "quantal" test because it is measures an effect that "occurs" or "does not occur".

Who invented the idea of an LD50?

- In 1927, J.W. Trevan attempted to find a way to estimate the relative poisoning potency of drugs and medicines used at that time. He developed the LD50 test because the use of death as a "target" allows for comparisons between chemicals that poison the body in very different ways. Since Trevan's early work, other scientists have developed different approaches for more direct, faster methods of obtaining the LD50.

半数致死浓度(LC50)：即能引起所观察个体半数死亡所需的浓度。

单位：mg/m^3和mg/l(水)来表示。

半数耐受限量/Median tolerance limit, TLm:指在一定时间内一群水生生物中50%的个体能够耐受的某种环境化学污染物在水体中的浓度，单位为mg/l

最小致死量：指仅引起一群个体个体中个别个体死亡的最低剂量。低于此计量则不引起个体死亡。

最大耐受量：这在一群个体中不引起死亡的某化学物的最高剂量。

What does LC50 mean?

- LC stands for "Lethal Concentration". LC values usually refer to the concentration of a chemical in air but in environmental studies it can also mean the concentration of a chemical in water.
- For inhalation experiments, the concentration of the chemical in air that kills 50% of the test animals in a given time (usually four hours) is the LC50 value.

2. 半数效应剂量

指外源化学物引起机体某项生物效应发生50%改变所需的剂量。

3. 3、最小有作用剂量/*minmal effect level*

最小有作用剂量 即在一定时间内，一种外来化合物按一定方式或途径与机体接触，能使某项观察指标开始出现异常变化或使机体开始出现损害作用所需的最低剂量，也可称为中毒阈剂量，或中毒阈值。

例如某种有机磷化合物在大鼠(*wistar*品系)经给予3个月，全血胆碱酯酶活力降低50%的最大无作用剂量为10mg/kg体重。

4、最大无作用剂量(*maximal no-effect level*)

最大无作用剂量：在一定时间内，一种外来化合物按一定方式或途径与机体接触，根据现今的认识水平，用最灵敏的试验方法和观察指标，亦未能观察到任何对机体的损害作用的最高剂量。

最大无作用剂量的确定系根据亚慢性毒性或慢性毒性试验的结果，是评定外来化合物对机体损害作用的主要依据。

What are some other toxicity dose terms in common usage?

- **LD01 Lethal dose for 1% of the animal test population**
- **LD100 Lethal dose for 100% of the animal test population**
- **LDLO The lowest dose causing lethality**
- **TDLO The lowest dose causing a toxic effect**

How are LD/LC50 tests done?

- In nearly all cases, LD50 tests are performed using a pure form of the chemical. Mixtures are rarely studied.
- The chemical may be given to the animals by mouth (oral); by applying on the skin (dermal); by injection at sites such as the blood veins (i.v.- intravenous), muscles (i.m. - intramuscular) or into the abdominal cavity/腹腔 (i.p. - intraperitoneal).

The LD50 value obtained at the end of the experiment is identified as the LD50 (oral), LD50 (skin), LD50 (i.v.), etc., as appropriate. Researchers can do the test with any animal species but they use rats or mice most often. Other species include dogs, hamsters, cats, guinea-pigs, rabbits, and monkeys. In each case, the LD50 value is expressed as the weight of chemical administered per kilogram body weight of the animal and it states the test animal used and route of exposure or administration; e.g., LD50 (oral, rat) - 5 mg/kg, LD50 (skin, rabbit) - 5 g/kg. So, the example "LD50 (oral, rat) 5 mg/kg" means that 5 milligrams of that chemical for every 1 kilogram body weight of the rat, when administered in one dose by mouth, causes the death of 50% of the test group.

If the lethal effects from breathing a compound are to be tested, the chemical (usually a gas or vapour) is first mixed in a known concentration in a special air chamber where the test animals will be placed. This concentration is usually quoted as parts per million (ppm) or milligrams per cubic metre (mg/m3). In these experiments, the concentration that kills 50% of the animals is called an LC50 (Lethal Concentration 50) rather than an LD50. When an LC50 value is reported, it should also state the kind of test animal studied and the duration of the exposure, e.g., LC50 (rat) - 1000 ppm/ 4 hr or LC50 (mouse) - 5mg/m3/ 2hr.

Which LD50 information is the most important for occupational health and safety purposes?

Inhalation and skin absorption are the most common routes by which workplace chemicals enter the body. Thus, the most relevant from the occupational exposure viewpoint are the inhalation and skin application tests. Despite this fact, the most frequently performed lethality study is the oral LD50. This difference occurs because giving chemicals to animals by mouth is much easier and less expensive than other techniques. However, the results of oral studies are important for drugs, food poisonings, and accidental domestic poisonings. Oral occupational poisonings might occur by contamination of food or cigarettes from unwashed hands, and by accidental swallowing.

How do I compare one LD50 value to another and what does it mean to humans?

- In general, the smaller the LD50 value, the more toxic the chemical is. The opposite is also true: the larger the LD50 value, the lower the toxicity.
- The LD50 gives a measure of the immediate or acute toxicity of a chemical in the strain, sex, and age group of a particular animal species being tested. Changing any of these variables (e.g., type animal or age) could result in finding a different LD50 value. The LD50 test was neither designed nor intended to give information on long-term exposure effects of a chemical.

Once you have an LD50 value, it can be compared to other values by using a toxicity scale. Confusion sometimes occurs because several different toxicity scales are in use. The two most common scales used are the "Hodge and Sterner Scale" and the "Gosselin, Smith and Hodge Scale". These tables differ in both the numerical rating given to each class and the terms used to describe each class. For example, a chemical with an oral LD50 value of 2 mg/kg, would be rated as "1" and "highly toxic" according to the Hodge and Sterner Scale (see Table 1) but rated as "6" and "super toxic" according to the Gosselin, Smith and Hodge Scale (see Table 2). It is important to reference the scale you used when classifying a compound.

It is also important to know that the actual LD50 value may be different for a given chemical depending on the route of exposure (e.g., oral, dermal, inhalation). For example, some LD50s for dichlorvos/敌敌畏, an insecticide commonly used in household pesticide strips, are listed below:

Oral LD50 (rat): 56 mg/kg

Dermal/皮肤 LD50 (rat): 75 mg/kg

Intraperitoneal/腹腔 LD50: (rat) 15 mg/kg

Inhalation LC50 (rat): 1.7 ppm (15 mg/m3); 4-hour exposure

Oral LD50 (rabbit) 10 mg/kg

Oral LD50 (pigeon:): 23.7 mg/kg

Oral LD50 (rat): 56 mg/kg

Oral (mouse): 61 mg/kg

Oral (dog): 100 mg/kg

Oral (pig): 157 mg/kg

Table 1: Toxicity Classes: Hodge and Sterner Scale					
		Routes of Administration			
		Oral LD50	Inhalation LC50	Dermal LD50	
Toxicity Rating	Commonly Used Term	(single dose to rats) mg/kg	(exposure of rats for 4 hours) ppm	(single application to skin of rabbits) mg/kg	Probable Lethal Dose for Man
1	Extremely Toxic	1 or less	10 or less	5 or less	1 grain (a taste, a drop)
2	Highly Toxic	1-50	10-100	5-43	4 ml (1 tsp)
3	Moderately Toxic	50-500	100-1000	44-340	30 ml (1 fl. oz.)
4	Slightly Toxic	500-5000	1000-10,000	350-2810	600 ml (1 pint)
5	Practically Non-toxic	5000-15,000	10,000-100,000	2820-22,590	1 litre (or 1 quart)
6	Relatively Harmless	15,000 or more	100,000	22,600 or more	1 litre (or 1 quart)

Table 2: Toxicity Classes: Gosselin, Smith and Hodge		
Probable Oral Lethal Dose (Human)		
Toxicity Rating or Class	Dose	For 70-kg Person (150 lbs)
6 Super Toxic	Less than 5 mg/kg	1 grain (a taste - less than 7 drops)
5 Extremely Toxic	5-50 mg/kg	4 ml (between 7 drops and 1 tsp)
4 Very Toxic	50-500 mg/kg	30 ml (between 1 tsp and 1 fl ounce)
3 Moderately Toxic	0.5-5 g/kg	30-600 ml (between 1 fl oz and 1 pint)
2 Slightly Toxic	5-15 g/kg	600-1200 ml (between 1 pint to 1 quart)
1 Practically Non-Toxic	Above 15 g/kg	More than 1200 ml (more than 1 quart)

Can animal LD50 data be applied to man?

- **In general, if the immediate toxicity is similar in all of the different animals tested, the degree of immediate toxicity will probably be similar for humans. When the LD50 values are different for various animal species, one has to make approximations and assumptions when estimating the probable lethal dose for man. Tables 1 and 2 have a column for estimated lethal doses in man. Special calculations are used when translating animal LD50 values to possible lethal dose values for humans. Safety factors of 10,000 or 1000 are usually included in such calculations to allow for the variability between individuals and how they react to a chemical, and for the uncertainties of experiment test results.**

How should an LD50 value be used?

The LD50 can be used:

- **As an aid in developing emergency procedures in case of a major spill or accident.**
- **To help develop guidelines for the use of appropriate safety clothing and equipment. For example, if the dermal LD50 value for a chemical is rated as extremely toxic, it is important to protect the skin with clothing, gloves (etc.) made of the right chemical-resistant material before handling. Alternatively, if a chemical has an inhalation LC50 value which indicates that it is relatively harmless, respiratory protective equipment may not be necessary (as long as the oxygen concentration in the air is in the normal range - around 18%).**
- **For the development of transportation regulations.**

As an aid in establishing occupational exposure limits.
As a part of the information in Material Safety Data Sheets.
Remember, the LD50 is only a ball park figure so that lethal toxicity can be compared. It says nothing about levels at which other acute toxic, but non-lethal, effects might occur.

The LD50 is only one source of toxicity information. For a more thorough picture of the immediate or acute toxicity of a chemical, additional information should be considered such as the lowest dose that causes a toxic effect (TDLO), the rate of recovery from a toxic effect, and the possibility that exposure to some mixtures may result in increasing the toxic effect of an individual chemical.

Where can I find LD50 and LC50 values

- The largest, single collection of LD50 and LC50 values is in the database Registry of Toxic Effects of Chemical Substances (RTECS) that is available by subscription on CD-ROM and on the Internet. Two other databases available from CCOHS, CHEMINFO and the Hazardous Substances Data Bank?(HSDB). Both of these are on the CHEMpendium CD-ROM; CHEMINFO is also accessible on the Internet.
- http://www.ccohs.ca/products/cdrom/c2.html

(六)、效应和反应/effect and response

- **1. What is effect?**

- **2. What is response?**

（七）、剂量效应关系和剂量反应关系

- **1. What is dose-effect relationship?**
- **2. What is dose-response relationship?**

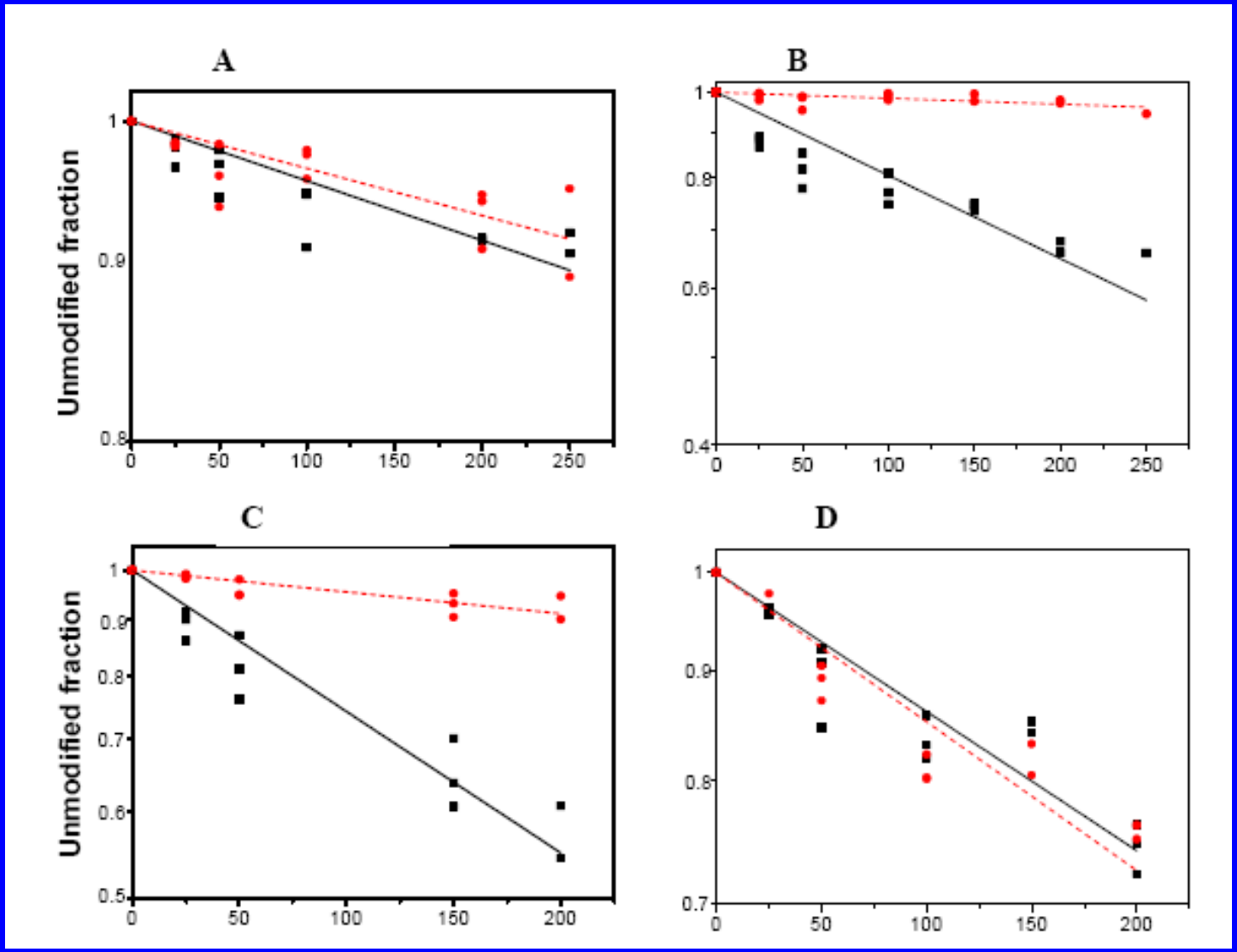
- 机体内出现的某种损害作用，如果肯定是某种外来化合物所引起，则必须存在明确的剂量效应或剂量反应关系，否则不能肯定。

1.

剂量效应和剂量反应关系都可以用曲线表示，即以表示效应强度的计量单位或表示反应的百分率或比值为纵座标，以剂量为横座标，绘制散点图，可得出一条曲线。

在一般情况下，剂量效应或剂量反应曲线有下列基本类型：

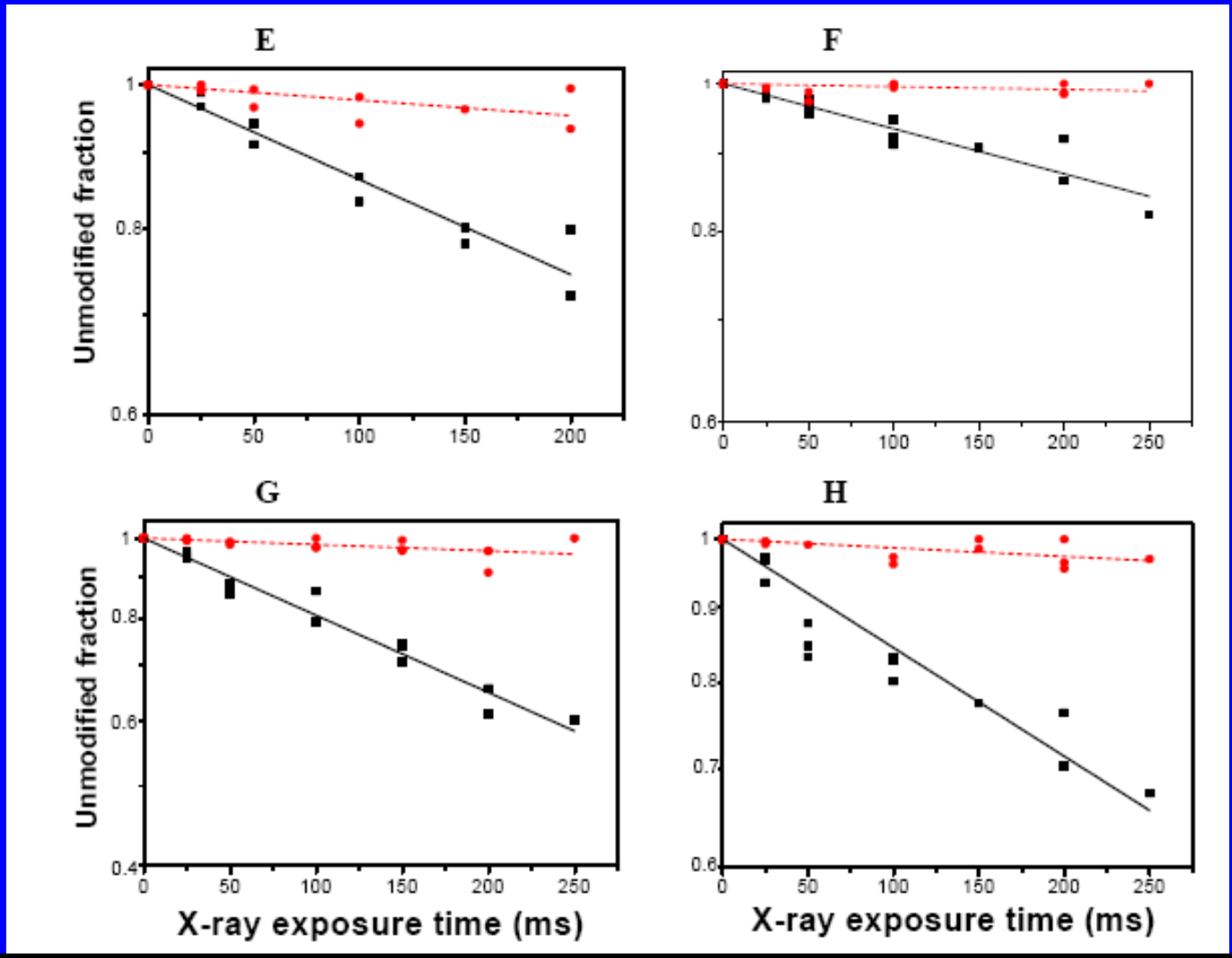
1、直线型 效应或反应强度与剂量呈直线关系；随着剂量的增加，效应或反应的强度也随着增加，并成正比关系。但在生物机体内，此种直线关系较少出现，仅在某些体外实验中，在一定的剂量范围内存在。



Dose Response curve

Rutao Liu, et.al., Biochem. 2003,42:13447-12454

剂量效应或剂量反应曲线有下列基本类型： 1、直线型



Dose Response curve

Rutao Liu, et.al., Biochemistry 2003, 42:13447-12454

2、抛物线型：

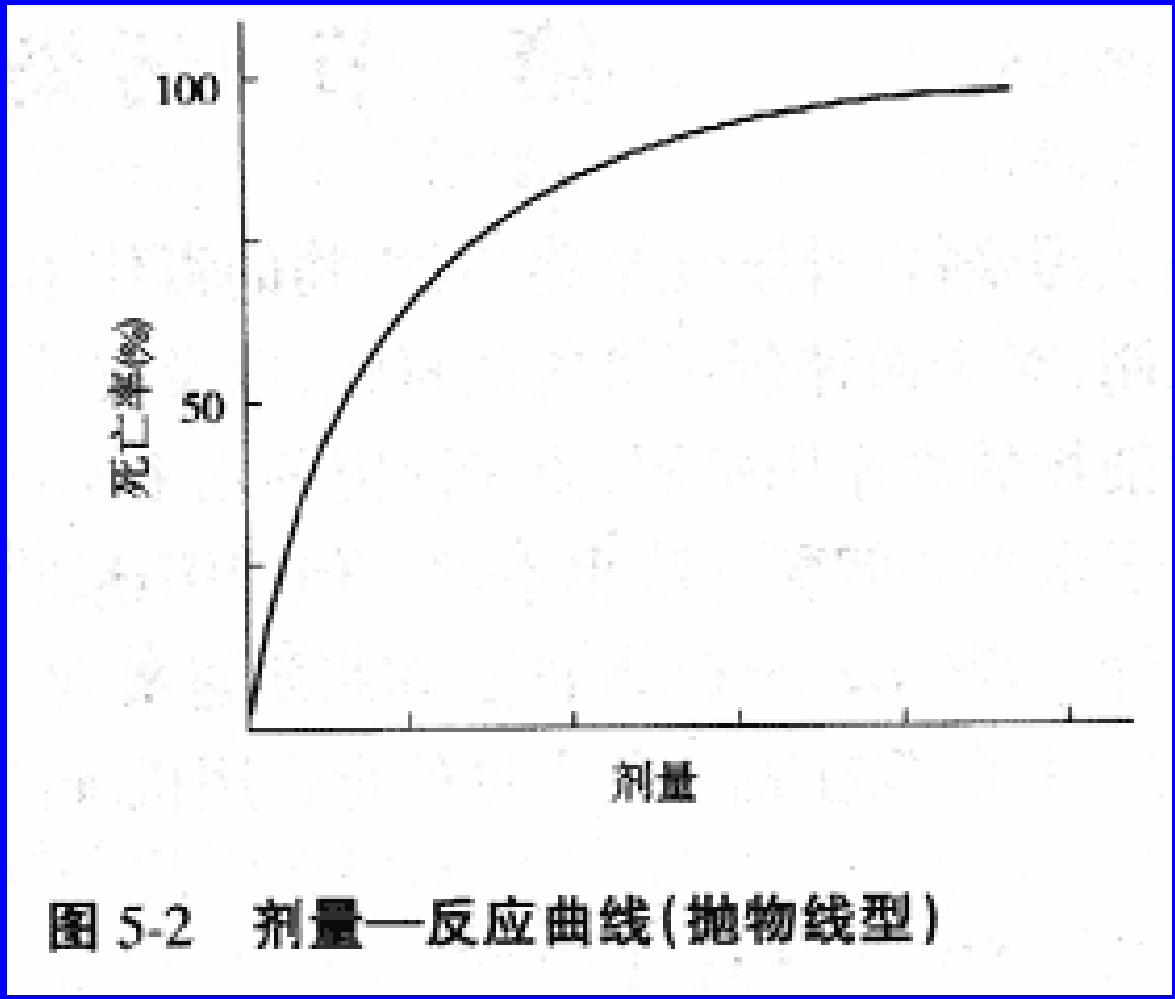


图 5-2　剂量—反应曲线(抛物线型)

3、S－状曲线

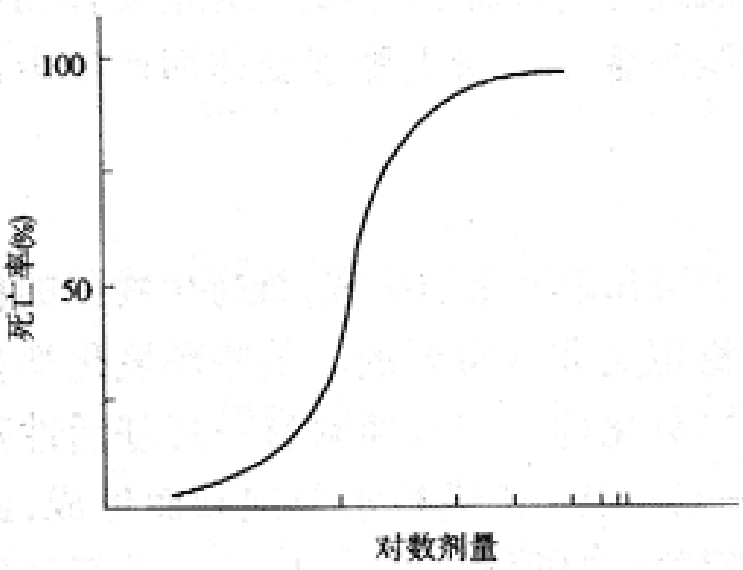


图 5-3　剂量—反应曲线(S 形线型)

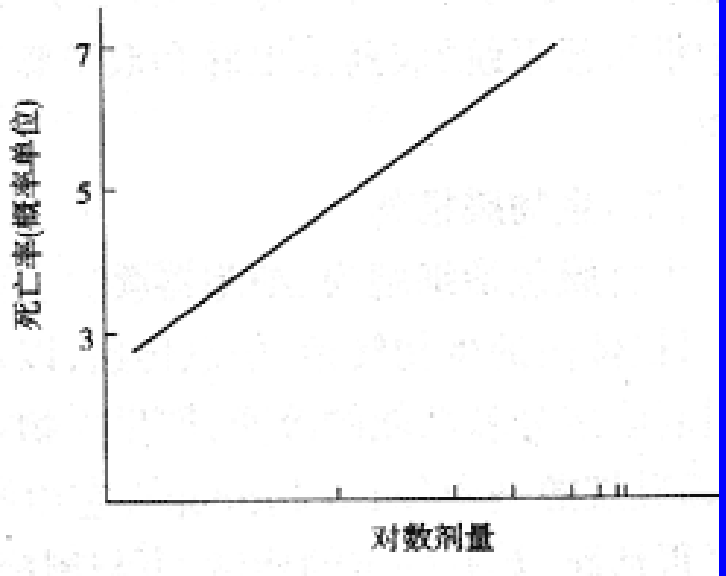


图 5-4　剂量—反应曲线

二、毒性作用的类型/Types of toxicological effects

- **Local and systemic effects**
  - **Local: contact on skin, respiratory tract, eg, HCl, Cl2, ammonium, caustic chemical**
  - **Systemic: absorbed and distributed, but focused on target organs, eg., DDT on Central Nerve System**

(二) 速发和迟发毒性作用

Immediate effect and delayed effects

Immediate: cyanide poisoning/氰化物中毒

Delayed: smoking exposure (carcinogen), radiation

(三)、可逆和不可逆毒性作用

Reversible and irreversible effects

Reversible: disappearance after cessation of exposure, eg., HCl, ammonium

Irreversible: persistence or progress after cessation of exposure, eg., carcinoma, mutations, damage to neurons, and liver cirrhosis, tetrodotoxin on muscles.

Example: insecticide on cholinerase,

Reversibility vs concentration and exposure time

(四)、变态反应/allergic reaction

What is allergic reaction?

What is the characteristic of allergic reation?

(五)、特异体质反应/idiosyneratic reaction

Whatis idiosyneratic reaction?

三、环境化学物的联合毒性作用/combined toxic effect

1. What is the combined toxic effect?

2. Introduction on the types of combined toxic effect:

(1).相加作用/additional joint action

Definition:

(2). 协同作用/synergistic joint action

Definition:

3. 增强作用：**potentiation**

4. 拮抗作用：**atagonistic joint action**

5. 独立作用/**Independent joint action**

(二) 联合作用类型的评定/Identification of the combined toxic effect

评定方法：

1. 联合作用系数法：

联合作用系数**(K)=**混合物的预期**LD50/**混合物的实测**LD50**

混合物**LD50**预期值的计算公式：

1/混合物预期值的**LD50 =a/A**的**LD50 +b/B**的**LD50 +……+n/N**的**LD50**

Table 3-1 联合作用系数**(K)**和联合作用类型

	为拮抗作用	为相加作用	为协同作用
Smyth法	**<0.4**	**0.4-2.7**	**>2.7**
Keplinger法	**<0.57**	**0.57-1.75**	**>1.75**

2. 等效应线图法

(本法只能评定两种化合物的联合作用**)**

方法原理：

(1). 制成等效应线。

(2).求出此混合物的**LD50**。

(3).评价联合作用的类型。

联合作用的类型的评价标准：

交点在可信限上下线之间，为相加作用；落在可信限的下线以下为协同作用；落在可信限上限以上为拮抗作用。

联合作用研究进展的参考文献：

顾兵，王心如，联合作用特征的评价，中国工业医学杂志，**2000,13(1): 55-58**

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四、毒性作用机理/toxicity mechanism

毒性作用的类型/Types of toxicity mechanism：

1. 干扰正常受体-配体的相互作用
2. 细胞膜损伤
3. 干扰细胞内钙稳定
4. 干扰细胞能量的产生
5. 自由基与脂质过氧化

6. 环境化学物与生物大分子结合

(1). 与核酸结合

(2). 与蛋白质、酶结合

(3). 与脂质结合

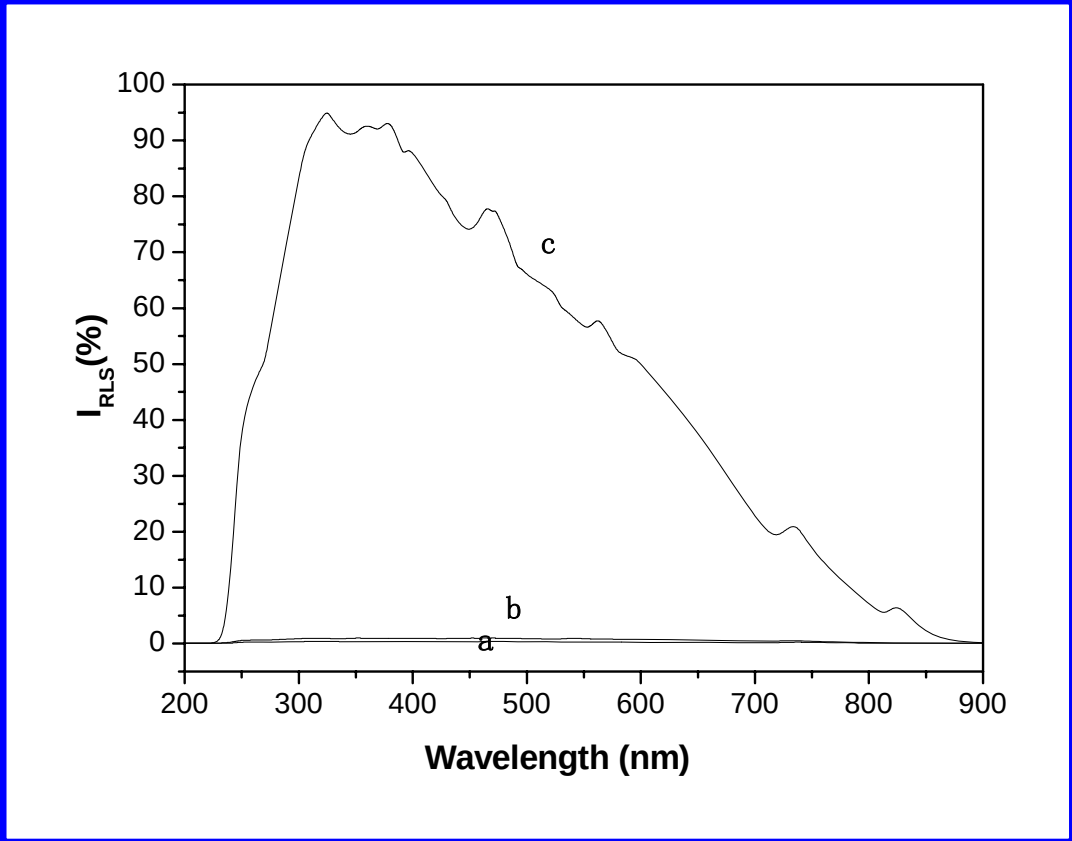


Fig. 1 Resonance light spectra of BSA, SDBS and after their toxic interaction

Fig.1 BSA，SDBS及其毒性作用后的共振光散射光谱图

a：SDBS b：BSA c：BSA+SDBS

BSA：1×10-6mol/L SDBS：1×10-4mol/L Buffer：BR pH＝3.2

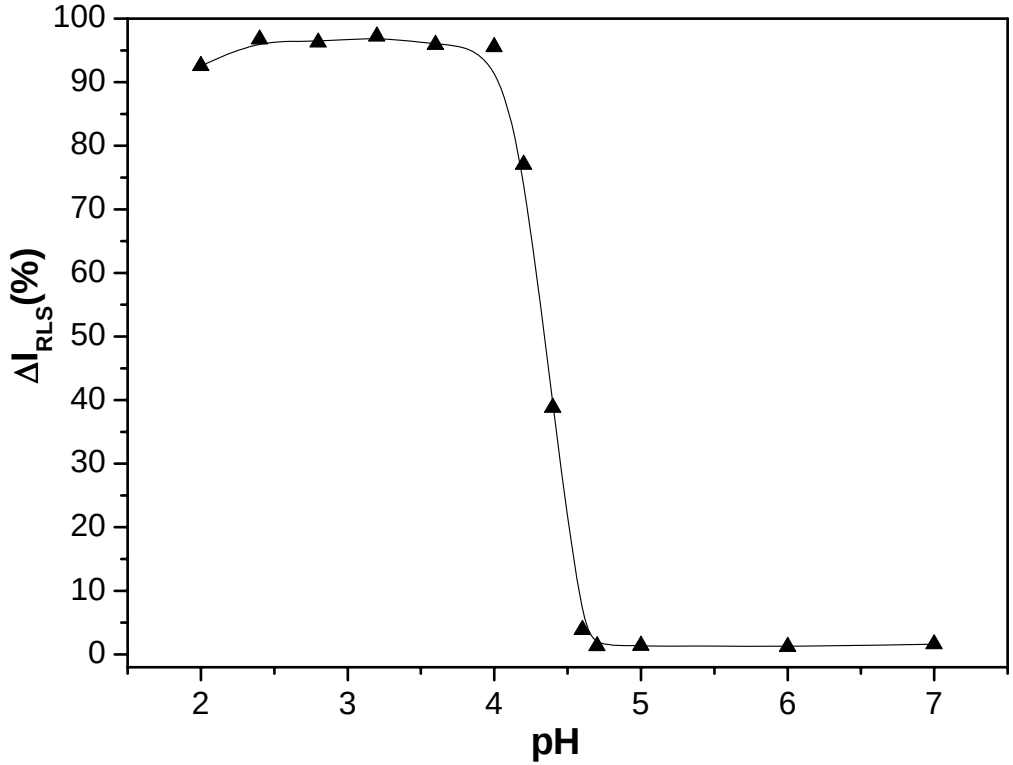


Fig.3　pH对体系RLS强度的影响

BSA：1×10-6mol/L SDBS：1×10-4mol/L Buffer：BR pH3.2

- **7.** 选择性细胞致死
- 有些外源化学物对某种组织器官的细胞有选择性致死毒性。

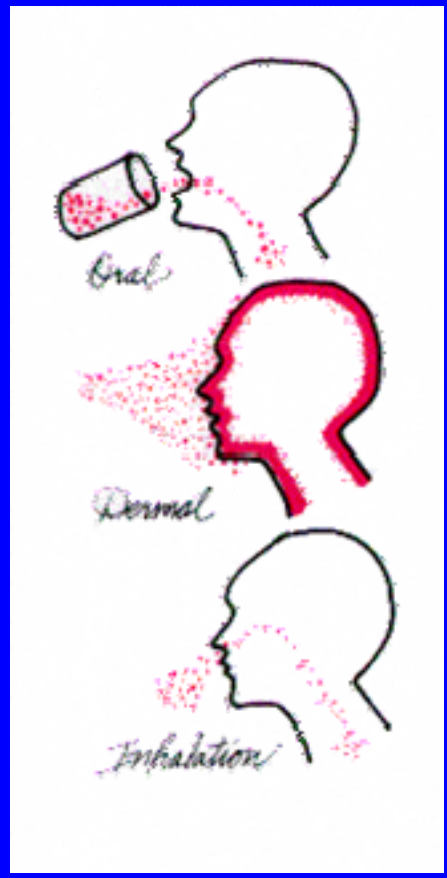
- **8.** 非致死性遗传改变
-

作业

- １．环境化学物毒性作用机理的类型有哪些？
- ２．已知化合物A和化合物B的LD50分别为15.30±0.12mg/kg, 25.68±0.21mg/Kg, 其等剂量混合物的LD50为19.8mg/kg。试用等效应曲线法来判定这两种化污染物联合毒性作用的类型。

第二节 影响毒性作用的因素

- **Please!**



第二节影响毒性作用的因素**Factors of toxic effects**

1. 环境化学物的结构和性质/Factors that can alter the toxic effects of toxicants

2. 机体状况/Animal hosts

3. 接触条件/ Chemical interactions

4. 环境因素/ Environmental

一、环境化学物的结构和性质

(一)结构与毒性

目前已知的部分规律：

1. 同系物的碳原子数
2. 烃基：
3. 分子饱和度：
4. 卤素取代：
5. 羟基：
6. 酸基和酯基
7. 胺基：

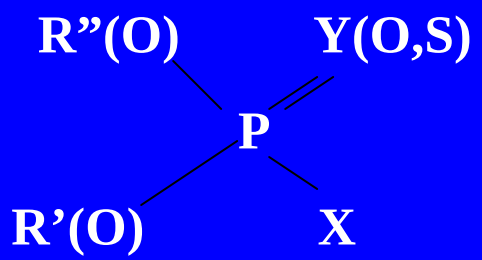
8. 构型

机体内的酶对化学物质的构型有高度特异性。

(1). 同分异构体：

(2). 旋光异构体

9. 有机磷化合物的结构与毒性



(二)物理性质与毒性/Physical properties and toxicity

1. 脂/水分配系数：

2. 电离度：

3. 挥发度和蒸汽压：

4. 分散度：

5. 纯度：

二、机体状况/Animal host factors

- Species, strain and individual
  - Toxicant are almost universally toxic to all animals

（一）种属和个体差异: **Species and individual difference**

Differences exists among species, eg.,

Hexobarbital/环己烯巴比妥 induced sleeping time: enzyme activities

Ethylene glycol: cat>rat>rabbit, proportional to oxalic content

Aniline/苯胺类化合物的毒性：

Cat and dog: o-aminophenol, more toxic

Rat and hamster: p-aminophenol, less toxic

2-naphthylamine2-萘胺:

Dog and human: bladder tumors膀胱癌

Rat, rabbit or guinea/豚鼠 pig: free of tumors

- Acetylaminofluorene(AAF):乙酰氨基芴
  - All animal but guinea pig/豚鼠: carcinogenic
- Squill／螳螂虾
  - Rat: rodenticide/灭鼠剂, no vomitting
  - Humans and other mammals: not toxic, vomitting

- Differences exists among strains / 疲劳, eg.,
  - All toxicants could induce changes in all strains
  - Tumorigenesis:
    - Sprague-Dawley: estrogen / 雌性激素 level higher—higher rate
    - F344 rat: estrogen level lower—lower rate
  - Adverse responses among the same strain: nitrosamines/亚硝胺, decalin十氢化萘, hydroquinone,/对苯二酚， CCl_4, etc.
    - Male: more susceptible

- Differences exists among individuals, eg.,
  - Acetyltransferase/乙酰基转移酶:
    - Slow reaction: deficient —accumulation of isoniazid/异烟肼(一种抗结核药物)—peripheral neuropathy
    - fast reaction: larger doses---hepatic damage
  - Plasma cholineterase: 血浆维生素B转移酶
    - Low level: prolonged muscular relaxation and apnea
  - Glucose-6-phosphate dehdrogenase: 葡萄糖磷酸脱氢酶
    - Deficiency: hemolytic anemia/溶血性贫血

(二) Sex, hormone status, and pregnancy

- Sexes
  - Barbiturates / 巴比妥酸盐
    - Female rat: prolonged sleep than in males
    - Male rat: short duration---liver microsomal enzymes:
  - Aminopurine/氨基嘌呤
    - Male: faster demethylation, less susceptible,
  - Sulfanilamide/磺胺
    - Male: faster acetylation, less susceptible

- Parathion / 对硫磷: organophosphorus
  - Female rat: faster metabolism, more toxic, more susceptible
- Chloroform / 氯仿
  - Male: nephrotoxic, higher concentration of cytochrome P-450
  - Female: not toxic
- Nicotine / 烟碱:
  - Male mouse: more toxic

- Digoxin / 地高辛(一种强心剂):
  - Male dog: more toxic
- Dinitrophenol:二硝基酚
  - Female: more toxic
- Benzene: 苯
  - Female rabbit: more toxic

三、接触条件/contact conditions

(一) 接触途径

生病时，有些即可口服又可注射的药为什么要打吊瓶，而非口服？

(二) 溶剂：

(三) 毒物浓度与体积

(四) 交叉接触

四、环境因素/Environmental factors

1、气温/Temperature

2、气湿

高温气湿使环境化学物镜皮肤吸收的速度加快。

3、气压/pressure

气压可引起某些化学物毒性作用的变化；

作业：

3. 判断下列化合物毒性的大小顺序：

（1） A.邻硝基苯酚； B.间硝基苯酚； C.对硝基苯酚

（2） A.二氯乙烷； B.四氯乙烷； C.氯乙烷