Chapter III

Concern Levels and Recommended Toxicity Tests

A. Introduction

This chapter describes how FDA determines which toxicity tests are recommended to assess the safety of food additives (direct food additives and color additives used in food) that are proposed for new or expanded use. Chapter III B explains how these additives are assigned to levels of concern (see Chapter III B 1) that indicate their potential for posing significant health risks to humans, if approved. A substance is assigned to a Concern Level based on available toxicology information or on the substance's structural similarity to known toxicants (see Chapter III B 2) and on the estimated human exposure to the substance from its proposed use (see Chapter III B 3). As in the previous edition of these guidelines (1982), exposure is weighted more heavily than structure in assigning substances to Concern Levels (see Figure 1).

Chapter III C describes the toxicity tests recommended for assessing the safety of additives (direct food additives and color additives used in food) assigned to each Concern Level. Different minimum testing levels are recommended for compounds assigned to Concern Levels I, II and III (see Table 2 in Chapter III C 1). Because Concern Level III substances may present more significant health risks than substances assigned to Concern Levels I and II, more rigorous and longer-term toxicity testing is recommended to assess the safety of Concern Level III substances. (Note that some tests in the minimum set of toxicity tests recommended for compounds assigned to Concern Levels I, II and III have been changed from those recommended in the 1982 publication; these changes are summarized in Chapter I A 2.) Chapter III C 2 explains how the Agency develops additional testing recommendations for assessing the safety of direct food additives and color additives used in food proposed for new or expanded use. These tests augment the minimum set of toxicity tests, as appropriate; examples are provided.

Detailed guidelines for specific toxicity tests are not included in this chapter. However, guidelines for the conduct of short-term tests for genetic toxicity, acute toxicity tests, short-term toxicity tests with rodents and non-rodents, subchronic toxicity tests with rodents and non-rodents, one-year toxicity tests with non-rodents, carcinogenicity studies with rodents, combined chronic toxicity/carcinogenicity studies with rodents, and reproduction and developmental toxicity studies, can be found in Chapter IV C. Guidelines to assist the petitioner in developing strategies for assessing the metabolism and pharmacokinetics, immunotoxicity and neurotoxicity of food additives and color additives used in food can be found in Chapter V and recommended strategies for conducting human clinical trials with direct food additives and color additives used in food can be found in Chapter VI B.

III B. Concern Levels

1. Determining Concern Levels

In 1982, FDA introduced the concept of tiered testing requirements for obtaining information about the safety of direct food additives and color additives used in food. This concept is based on the assumption that the degree of effort expended to reduce uncertainty about the safety of a direct food additive or color additive used in food should relate in some logical way to the likelihood that the additive poses a health risk to the public.

In evaluating the toxicological safety of direct food additives and color additives used in food, two factors are of primary importance: the extent of human

exposure (dose) and the toxicological effects on various biological systems (nature of effect, target, magnitude of response per unit dose, etc.). These factors determine the extent of the Agency's initial concern about the safe use of an additive. The greater the Concern Level, the greater the potential for toxicity.

In the absence of toxicological information about a compound, potential toxicity can be evaluated on the basis of structural similarity to known toxicants (see Chapter III B 2). Information about a compound's potential toxicity and estimated human exposure from a designated use (see Chapter III B 3) are sufficiently useful to permit semi-quantitative categorization of direct food additives and color additives used in food into three broad initial Concern Levels. For example, high toxic potential and high exposure would result in a compound being assigned a high initial Concern Level (*i.e.* Concern Level III), and low toxic potential and low exposure would result in a compound being assigned a low initial Concern Level (*i.e.* Concern Level I). Thus, Concern Levels are relative measures of the degree to which the use of an additive may present a hazard to human health.

Available toxicology information can, of course, change the Concern Level to which an additive has been assigned and alter the recommended set of toxicity tests for the additive. Subsequent and final Concern Levels, therefore, may be different from the initial Concern Level, and will be based on estimated human exposure and actual information about the metabolism and toxicology of the compound. For example, an additive may be transformed by metabolic activity into a substance of greater potential toxicity, or a potentially toxic additive may be distributed or metabolized in a manner that protects the target tissue or organ from the toxic effects of the chemical (blood-brain barrier; placental barrier; metabolic deactivation).

The minimum set of recommended toxicity tests for each additive (*i.e.* direct food additives and color additives used in food) is determined by the initial Concern Level to which it is assigned (see Chapter III C 1). Recommended toxicity tests are designed to reduce uncertainty about the safety of direct food additives and color additives used in food that have been proposed for new or expanded use. In addition, these testing recommendations allow more resources to be concentrated on additives that present the highest probable risk to human health (*i.e.* Concern Level III substances); fewer resources per additive can be expended on additives where use levels and potential toxicity are minimal (*i.e.* Concern Level I substances). Such a system for development of toxicology information is expected to be more cost-effective than one in which all additives are made to undergo the same regimen of testing irrespective of any other considerations.

In general, the procedure for determining the initial Concern Level for a direct food additive or color additive used in food is as follows:

- On the basis of information about its molecular structure, an additive will be placed in one of three broad categories: Category C is for additives whose toxicological potential is considered to be high; Category A is for additives whose toxicological potential is considered to be low; and Category B is for additives whose toxicological potential is likely to be intermediate between Categories A and C (see Chapter III B 2).

- Human exposure to each additive will be estimated (see Chapter III B 3).

- Within each structure category (A, B, and C), estimated human exposure will determine the initial Concern Level to which each additive is assigned (see Figure 1 below).

The choice of three broad Concern Levels reflects the traditional division of toxicity studies into three broad classes based on duration of exposure to the test compound: Short-term, subchronic, and chronic. As the duration of the exposure increases, the lowest-effect dose and the types of effects observed usually are determined with increasing sensitivity. Similarly, as the Concern Level to which an additive has been assigned increases from I to III, the recommended duration of toxicity studies and exposure to the test compound also increase (see Chapter III C 1 and Table 2). As data from the minimum set of toxicity tests are obtained, the

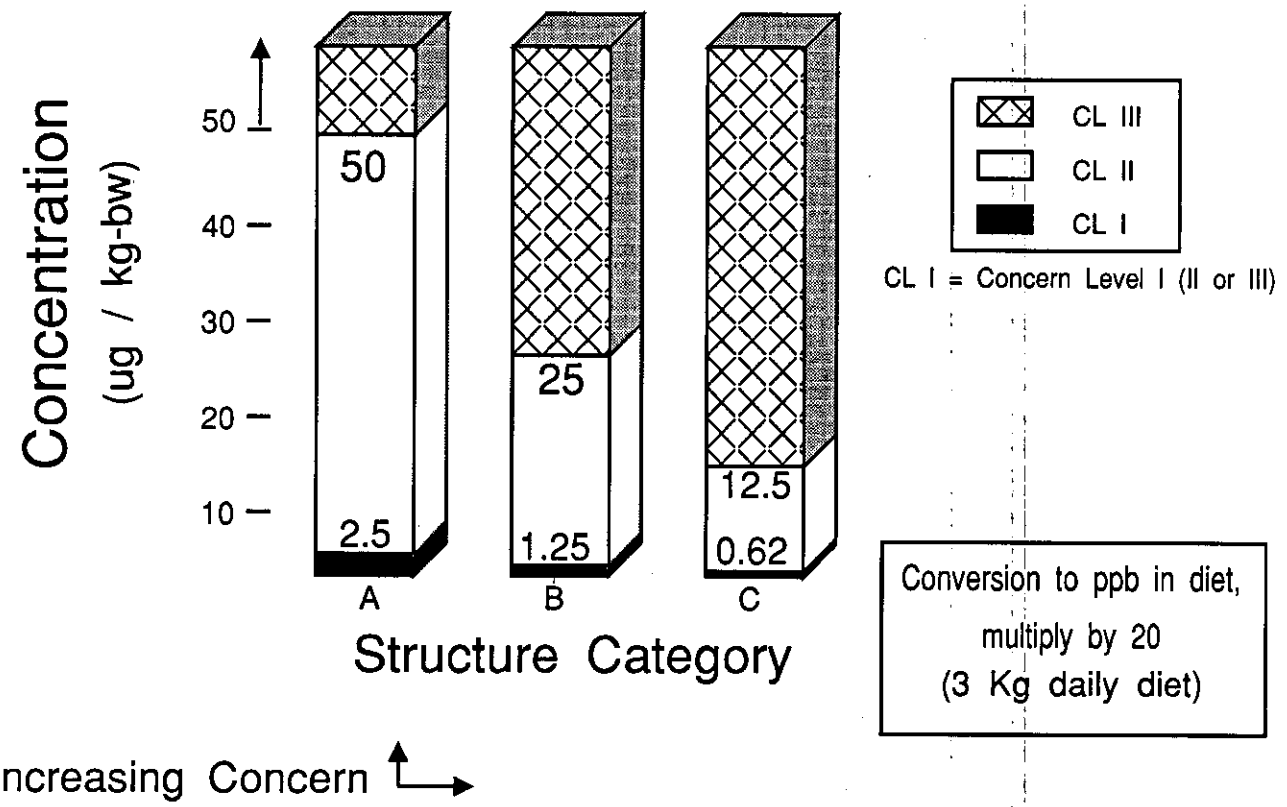
results can be used to refine or adjust the type, sensitivity, and rigor of subsequent tests, and therefore the precision of the estimate of an additive's toxicity.

Levels of exposure that define which substances in each Structure Category are assigned to Concern Levels I, II, and III (see Figure 1) were selected in 1982 on the basis of recommendations by experienced toxicology experts within CFSAN. While exposures may range over approximately 6 orders of magnitude, the structure category of the substance has the effect of only having the breakpoints for determining Concern Level assignments between structure categories A or B or between structure categories B or C. Structure category is allowed only this limited influence in determining minimum testing levels partly because of the considerable uncertainty still surrounding the use of chemical structure to estimate potential toxicity. [1]

As noted previously, a food or color additive is considered safe if there is a reasonable certainty that no harm will result from its use (see Chapter II C). The level of exposure for which there is a reasonable certainty of no harm usually can be extrapolated from data obtained from toxicity studies. Thus, for each toxic effect associated with a food or color additive, the degree of concern can be defined as the extent to which actual exposure is expected to exceed the acceptable daily intake (acceptable exposure) determined from toxicological information. Because the degree of concern is also a function of the nature of the toxic effect, information that indicates a more severe toxic effect (for example, irreversible and life-threatening effects) may increase the Concern Level of a substance, regardless of exposure.

Figure 1

Concern Levels as Related to Chemical Structure and Exposure



III B 2. Structure Category Assignment

a. Introduction

The toxic action of a compound is a consequence of the physical and chemical interaction of the compound or its active form with a critical molecular target--receptor, enzyme, DNA or other cellular constituent--within the living organism. Thus, it is reasonable to expect that the structure and associated physicochemical properties of a compound play an important role in its toxicity. This relationship between toxicity and chemical structure forms the basis for various systematic schemes and approaches developed over the years in attempts to estimate the toxic potential of untested chemicals or to prioritize chemicals for toxicity testing. [2-6]

In recent years, a number of computer-assisted, structure-activity relationship (SAR) models have been developed for predicting or estimating the toxicity of untested compounds. A general approach to developing such a model is to derive a correlation equation that relates structural features and physicochemical parameters of compounds to the toxicological endpoint of interest. The correlation equation is based upon a database assembled from a series of structurally related compounds or a set of heterogeneous structures. [7] The parameters (or variables) commonly used in SAR modeling fall into four major categories: topological, geometric, electronic and physicochemical, [8] as illustrated below:

- Topological parameters: counts of atoms and bonds, molecular weight, counts of rings and ring atoms, presence or absence of selected functional groups and substructural fragments;
- Geometric parameters: molecular size and shape;
- Electronic parameters: partial charge, dipole moments and bond strength; and
- Physicochemical parameters: partition coefficient.

Using this general approach, Enslein and co-workers, [9-14] Jurs and co-workers, [15-22] and Klopman and Rosenkranz [23-29] have constructed SAR models for a number of toxicological endpoints, such as acute toxicity in rodents (*i.e.* LD_{50}), carcinogenicity, mutagenicity in *Salmonella typhimurium*, and teratogenicity.

The Agency recognizes that certain chemical structures bear some relationship to biological activity. While these SAR models hold great promise for specific applications in the future, they are subject to several major limitations at this time. Because of these limitations, the Agency believes that information about such relationships should be used only to guide recommendations about the acquisition of toxicological data, and not as a substitute for such data. Acting on this premise, the Agency continues to incorporate information on chemical structures into its recommendations about the initial level of testing recommended to demonstrate the toxicological safety of a direct food additive or a color additive used in food.

The purpose of this chapter is to provide a general guideline whereby a chemical that has been proposed as a direct additive or a color additive for use in food can be assigned to a Chemical Structure Category based on substructural features--specifically, the presence or absence of chemical groups that have been associated with certain types of toxicity. This information will be integrated with data on predicted human exposure to determine the potential for toxicity, and thus the recommended initial level of toxicity testing for the proposed additive.

The guidelines provided in this Chapter are not intended to be comprehensive or as a rigid set of rules. Substructural fragments and functional groups are illustrative of those groups identified in the Structure Categories Section below. The initial, usually temporary, Concern Level to which a substance has been assigned is based on its structure category assignment (see Chapter III B 2 b) and the estimated human exposure to the substance from its petitioned use (see Chapter III B 3). This initial Concern Level will be modified during the review process based on chemical or biological information, such as: 1) the functional groups of known or predicted metabolites of the additive are judged to be of more or less concern than the structure of the additive; 2) there is evidence of potential bioaccumulation of the additive or its metabolites; 3) there is unequivocal evidence that the additive is poorly or not absorbed; or 4) qualitative or quantitative information is available on secondary component(s) or contaminants.

b. Structure Category Assignment of Additives

The initial step in assigning a proposed direct food or color additive to its correct Structure Category is to identify its complete chemical structure(s) and functional group(s). A direct food additive or color additive used in food may be a single chemical (arbitrarily defined as a chemical that is ≥ 90% pure), or a compound that is a mixture of two or more chemicals. Each chemical component in an additive is evaluated for the presence of one or more functional groups. Based on this information, the additive under consideration can be placed in the appropriate Chemical Structure Category. Structure Categories are divided into three classes of potential for toxicological significance (*e.g.* Categories A, B, and C), with Category A having the least potential for toxicity and Category C having the highest potential for toxicity.

This Chapter is an updated version of the "Chemical Structure Category Section" in the 1982 Agency guidelines. While the major groups of chemical structure categories presented in 1982 Agency guidelines have remained unchanged, the majority of these categories have been subdivided into smaller groups of chemicals that share common functional groups. For those petitioners who would like additional information on the assignment of chemicals to different structural categories, the Agency has a supplemental document entitled "*Structure Category Assignments of Chemicals in the Priority-Based Assessment of Food Additive Database*" available upon request.

Structure Category A

i. Structure Category A Chemicals

In general, **Structure Category A** includes compounds with chemical structures (substructural fragments and functional groups) believed to be of low toxic potential. It includes substances that are normal cellular constituents (*e.g.* certain fats and carbohydrates), but it excludes amino acids, proteins and certain intermediates of lipid and carbohydrate metabolism. The aliphatic organic chemicals in this category have relatively simple structures that are saturated. Inorganic chemicals in this category are certain endogenous salts of alkali metals (*e.g.* sodium and potassium) and alkaline-earth metals (*e.g.* calcium and magnesium).

Chemicals in **Structure Category A** can be divided into three general groups, including: 1)aliphatic hydrocarbons (saturated + un-functionalized or mono-functional), 2) fats and carbohydrates, and 3) inorganic chemicals.

Aliphatic Hydrocarbons

- Aliphatic hydrocarbons: un-functionalized and non-cyclic {C=2 to 30}: Group includes saturated straight- and branched-chain alkanes.

alkanes: $\mathbf{CH_3\text{-}(CH_2)_n\text{-}CH_3}$

Structure Category A

- Aliphatic hydrocarbons: un-functionalized, saturated and mono-cyclic {C=6 to 20}:

e.g. cyclohexane

- Aliphatic hydrocarbons: mono-functional, saturated and non-cyclic {C=2 to 30}: Group includes mono-functional:

aliphatic acids ($R''\text{-}COOH$) and alcohols ($R''\text{-}OH$),
aliphatic aldehydes ($R''\text{-}CH{=}O$) and esters ($R''\text{-}COO\text{-}R''$),
aliphatic ethers ($R''\text{-}CH_2\text{-}O\text{-}CH_2\text{-}R''$) and ketones ($R''\text{-}CO\text{-}R''$), and
aliphatic mercaptans ($R''\text{-}SH$).

- Aliphatic hydrocarbons: mono-functional, saturated and mono-cyclic {C=6 to 20}: Group includes mono-functional, mono-cyclic acids; mono-functional, mono-cyclic alcohols; mono-functional, mono-cyclic aldehydes; mono-functional, mono-cyclic esters; mono-functional, mono-cyclic ethers; mono-functional, mono-cyclic ketones; and mono-functional, mono-cyclic mercaptans.

Fats and Carbohydrates

- Fats, fatty acids, fatty acyl esters and their salts: Group includes: fats, unsaturated and saturated fatty acids and fatty acyl esters.

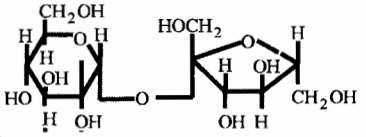
fats (*e.g.* butter esters, coconut and peanut oil),
unsaturated fatty acid (*e.g.* oleic acid: $CH_3\text{-}(CH_2)_7\text{-}CH{=}CH\text{-}(CH_2)_7\text{-}COOH$),
saturated fatty acid (*e.g.* caprylic acid: $CH_3\text{-}(CH_2)_6\text{-}COOH$), and
fatty acyl esters ($R'\text{-}COO\text{-}R''$).

- Intermediates and products of carbohydrate and lipid metabolism in humans:

intermediates of carbohydrate metabolism (*e.g.* citric acid) and
intermediates of lipid metabolism (*e.g.* lecithin)

- Simple and complex carbohydrates: Group includes carbohydrates which are components in the human diet, including: saccharides, oligosaccharides, and polysaccharides.

simple carbohydrates (*e.g.* gluconic acid: $HOOC\text{-}(CHOH)_4\text{-}CH_2\text{-}OH$ and sucrose)



complex carbohydrate (*e.g.* starch)

Inorganic Chemicals

- Endogenous (normal cellular constituents) inorganic salts: Group includes alkali metals (Na^+, K^+), alkaline-earth metals (Mg^{2+}, Ca^{2+}), simple ammonium salts (NH_4^+), hydrochloric acid, sodium hydroxide and anions (Cl^-, CO_3^{2-}, NO_3^-, PO_4^{3-}, and SO_4^{2-}).

e.g. sodium chloride NaCl

- Inert gases: Group includes certain inert gases [*e.g.* argon (Ar), helium (He) and nitrogen (N_2)]. It also includes carbon dioxide (CO_2) and elemental carbon.

ii. Structure Category B Chemicals

Structure Category B includes compounds with chemical structures that have been associated with adverse effects other than mutagenicity and carcinogenicity in animals or humans. **Structure Category B** also includes indeterminate structures and structures believed to have a potential for toxicity that is intermediate between structures in Structure Categories A and C. Chemicals in Structure Category B can be divided into four general groups, including: 1) aliphatic hydrocarbons (certain *mono-functional* and *saturated*, as well as *mono-functional* or *multi-functional*, *unsaturated* and *non-conjugated* chemicals); 2) amino acids, proteins and certain nitrogenous chemicals; 3) inorganic chemicals; and 4) mixtures of defined chemicals (with only Category A or B chemicals).

Aliphatic Hydrocarbons

- Aliphatic hydrocarbons: mono-functional hydrocarbons not listed in **Categories A & C**: Group includes:

mono-functional aliphatic acetals [$(R''O)_2$-CH-R''];
mono-functional glycol ethers
(*e.g.* ethylene glycol monomethyl ether HO-CH_2-CH_2-O-CH_3); and
methyl alcohol (CH_3-OH) and methyl esters (R''-COO-CH_3).

- Aliphatic hydrocarbons: mono-functional and mono-unsaturated: Group includes both cyclic and non-cyclic mono-functional and mono-unsaturated hydrocarbons.

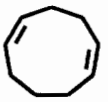
mono-functional and mono-unsaturated, non-cyclic hydrocarbons
(*e.g.* 2-hexene: CH_3-CH=CH-$(CH_2)_2$-CH_3)

mono-functional and mono-unsaturated, cyclic hydrocarbons
(*e.g.* cyclohexene)

- Aliphatic hydrocarbons: mono-functional and poly-unsaturated (& non-conjugated) {C=6 to 30}: Group includes both cyclic and non-cyclic mono-functional and polyunsaturated (non-conjugated) hydrocarbons.

mono-functional and poly-unsaturated (non-conjugated), non-cyclic hydrocarbons
(*e.g.* 1,4-pentadiene: HC_2=CH-CH_2-CH=CH_2)

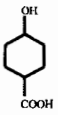
mono-functional and polyunsaturated (non-conjugated), cyclic hydrocarbons
(*e.g.* 1,5-cyclononene)



- Aliphatic hydrocarbons: multi-functional and saturated, mono-unsaturated or polyunsaturated (non-conjugated): Group includes: multi-functional, saturated, non-cyclic hydrocarbons; multi-functional, saturated, cyclic hydrocarbons; multi-functional, mono-unsaturated (or polyunsaturated & non-conjugated), non-cyclic hydrocarbons; multi-functional, mono-unsaturated (or polyunsaturated & non-conjugated) cyclic hydrocarbons. Examples of multi-functional chemicals included in this group are unsaturated carboxylic ethers and anhydrides, polyaldehydes and polyols.

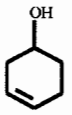
multi-functional, saturated, non-cyclic hydrocarbons
e.g. 2-hydroxypropionaldehyde: CH_3-CH(OH)-CHO

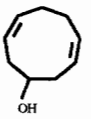
multi-functional, saturated, cyclic hydrocarbons
e.g. 4-hydroxycyclohexanoic acid



multi-functional, mono-unsaturated (or polyunsaturated and non-conjugated) non-cyclic hydrocarbons (*e.g.* 3-hexenol: CH_3-CH_2-CH=CH-CH_2-CH_2-OH)

multi-functional, mono-unsaturated (*e.g.* 3-cyclohexen-1-ol, *center*) or poly-unsaturated & non-conjugated cyclic hydrocarbons
(*e.g.* cyclonona-3,7-dien-1-ol, *right*)





Amino Acids, Proteins and Certain Nitrogenous Chemicals

- Amino acids: Group includes amino acids, unless they contain functional groups listed in Category C.

  e.g. alanine: CH_3-$CH(NH_2)$-COOH

- Proteins and polypeptides
  proteins (*e.g.* yeast protein extract) and
  polypeptides (*e.g.* protein hydrolysate)

- Certain nitrogenous chemicals: Group includes quaternary ammonium salts, alkylated ammonium compounds, and urea.

  e.g. quaternary ammonium salts $[(R'')_4N^+ \ X^-]$ and urea $[NH_2$-CO-$NH_2]$

Inorganic Chemicals

- Inorganic salts of Fe, Cu, Mn, Zn, and Sn: Category also includes simple iodide salts (*e.g.* sodium iodide), sulfur dioxide (SO_2) and silicates (*e.g.* $NaSiO_3$). Furthermore, this category includes organic salts of the same inorganic chemicals, so long as the metal is not covalently bonded to the organic substance (and the organic substance is <u>not</u> included in Category **C**).

  e.g. ferric sulfate: $Fe_2(SO_4)_3$

Mixtures

- Mixtures of chemicals: Group includes only mixtures of chemicals of defined composition, and all of the chemicals in the mixture must be assigned to Category A, Category B, or Categories A and B.

iii. <u>Structure Category C Chemicals</u>

In contrast to the two previous categories, **Structure Category C** contains compounds or metabolites that are structurally related to a reported mutagen or carcinogen, or chemicals that are structurally related to compounds demonstrated to produce carcinogenicity in humans or laboratory animals. A total of 55 individual subgroups of chemicals have been pooled into six major groups based upon the presence or absence of specific types of chemical functional groups, including:

- aliphatic (multi-functional & conjugated) alkene and alkyne hydrocarbons (with and without C and O functional groups);
- aromatic (mono- and polycyclic) hydrocarbons (mono- and multi-functional);
- aliphatic and aromatic (mono- and multi-functional) hydrocarbons with functional groups containing N, P and S atoms;
- heterocyclic chemicals (chemicals have a closed ring structure that contains one or more atoms within the ring that differ from carbon (*e.g.* nitrogen, oxygen and sulfur);
- inorganic and organometallic chemicals; and
- mixtures of chemicals (with Category C and unknown chemicals).

Structure category assignment of chemicals in Category C is relatively straightforward when the additive has only one functional group. When the additive has more than one specified functional group, a conservative approach is used by the FDA. Chemicals with more than one Category C functional group are assigned to all of the appropriate Category C functional groups.

Structure Category C

Aliphatic Alkenes & Alkynes

This group of chemical structure categories includes chemicals with relatively simple aliphatic and aromatic structures that are devoid of nitrogen, sulfur and phosphorus functional groups

- Aliphatic hydrocarbons: unsaturated (& conjugated) and non-aromatic: Group includes conjugated (non-cyclic and cyclic, but not aromatic) alkenes, aldehydes, and ketones. It also contains α,β-unsaturated (non-cyclic or cyclic, but not aromatic) carbonyl (having an *alpha* (α), *beta* (β) unsaturated double bonded carbon and oxygen {*e.g.* R''-CH=CH-C(O)-R''}) acids and esters. In addition, all conjugated non-cyclic and cyclic chemicals with an allyl fragment {*e.g.* CH_2=CH-CH_2-} are included in this group.

conjugated alkenes [R'-CH=CH-CH=CH-R'];
conjugated aldehydes [R'-CH=CH-CH=O] and ketones [R'-CH=CH-CO-R'];
α,β-unsaturated carbonyl acids [R'-CH=CH-COOH] and esters [R'-CH=CH-CO-O-R']; and chemicals with an allyl fragment (CH_2=CH-CH_2-).

- Alkynes:

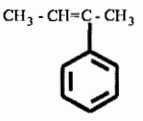
alkynes: R'-C≡C-R'

Aromatic Hydrocarbons

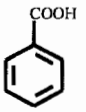
- Aromatic hydrocarbons: Group includes mono-aromatic hydrocarbons, including: mono-aromatic chemicals with or without alkyl functional groups; mono-aromatic chemicals with conjugated alkenes (including the allyl functional group); mono-aromatic α,β-unsaturated carbonyl acids and esters; mono-aromatic, conjugated aldehydes and ketones; mono-aromatic chemicals with the oxy functional group (*e.g.* methoxy, ethoxy, *etc.*); and mono-aromatic chemicals with one or more hydroxy (-OH) functional groups.

mono-aromatic benzene ± alkyl functional groups (*e.g.* benzene)

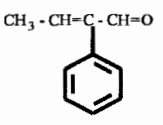
mono-aromatic conjugated alkene (*e.g.* 2-phenyl-2-butene)



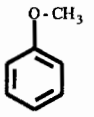
mono-aromatic α,β-unsaturated carbonyl (*e.g.* benzoic acid)



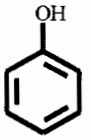
mono-aromatic, conjugated aldehydes and ketones (*e.g.* 2-phenyl-2-butenal)



mono-aromatics with the oxy functional group (*e.g.* anisole)

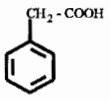


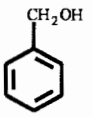
mono-aromatics with hydroxyl functional group (*e.g.* phenol)

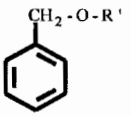


- Benzylic hydrocarbons: Group includes aromatic hydrocarbons with the benzylic functional group, including: benzylic acids, alcohols, aldehydes, and esters.

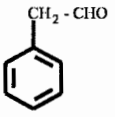
benzylic acid (*left*), benzyl alcohol (*center*), and benzyl ethers (*right*)

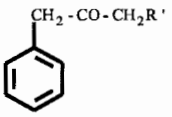


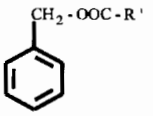




phenylacetaldehyde (*left*), benzyl ketones (*center*) and benzylic esters (*right*)

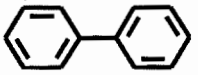




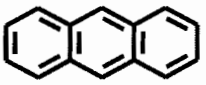


- Polycyclic aromatic hydrocarbons: Group includes:

biphenyl, aromatic hydrocarbons (*e.g.* biphenyl) and



polycyclicaromatic hydrocarbons [*e.g.* anthracene (*center*) and fluorene (*right*)]

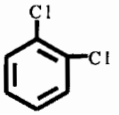


Aliphatic and Aromatic Chemicals Containing Halogen, Nitrogen, Phosphorus, and Sulfur Functional Groups

This group of chemical structure assignment categories includes chemicals that contain halogen, nitrogen, phosphorus, and sulfur chemical functional groups.

- Halogenated chemicals: Group includes:

aliphatic [R'-CH(X)-R'] and aromatic halides (*e.g.* 1,2-dichlorobenzene);



halocarbonyl acids (R'-CH(X)-COOH) and aldehydes (R'-CH(X)-CHO);
halocarbonyl amides (R'-CH(X)-CO-NH_2) and esters (R'-CH(X)-COO-R');
haloethers (*e.g.* α-alkyl haloether: R'-CH(X)-O-CH_2-R'); and
halohydrins (R'-CH(X)-CH_2-OH).

Nitrogen Functional Groups

Chemicals containing nitrogen functional groups include hydrazides, hydroxylamides, imines, un-substituted amides, and lactams; aliphatic and aromatic amines; nitro and nitroso groups; N-nitroso group; nitriles; azo and di-azo chemicals; azoxy chemicals; azide and triazene chemicals; hydrazines; carbamic acid esters; urea derivatives; guanidines; isocyanates; isothiocyanates; carbodiimides; and organic nitrates and nitrites. In contrast, heterocyclic chemicals that contain nitrogen within the ring structure are presented later in the section entitled "**Nitrogen Heterocyclic Chemicals**" along with other heterocyclic chemicals.

- Hydrazides; hydroxylamides and hydroxylamines; imines and hydroxylimines; and un-substituted amides:

  hydrazides [R'-CO-NH-NH_2];
  hydroxylamides (R'-CO-NH-OH) and hydroxylamines (R'-NH-OH);
  imines (R'-CH=NR') and hydroxylimines (R'-CH=N-OH); and
  unsubstituted amides (*e.g. primary*-amide R'-CO-NH_2).

- Aliphatic and aromatic amines: Group includes:

  1°-amines (R'-NH_2), 2°-amines (R'_2-NH) and 3°-amines (R'_3-N)

- Nitro and nitroso groups:
  nitro (R'-NO_2) and nitroso (R'-NO)

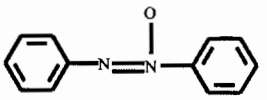
- N-nitroso group:
  N-nitroso: *e.g.* R'-NH-NO

- Nitriles:
  nitriles: R'-C≡N

- Azo and poly-azo chemicals:

  mono-azo (R'-N=N-R') and di-azo (R'-CH=N^+=N)

- Azoxy group:
  e.g. azoxybenzene



- Azides and triazenes:
  azides (R'-N=N^+=N^- or R'-N_3) and triazenes (R'-N=N-NH-R')

- Hydrazines:
  hydrazines: R'-NH-NH-R'

- Carbamic Acid Esters: Group includes:
  carbamic acid ester (R'-NH-C(O)-OR'),
  halogenated carbamic acid esters (R'-CH(X)-NH-C(O)-OR'), and
  thiocarbamic acid esters (R'-NH-C(S)-OR').

- Substituted ureas:
  substituted ureas: R'''-NH-CO-NH-R'''

- Guanidines:
  guanidines: NH_2-C(=NH)-NH-R'

- Isocyanates and cyanates:
  isocyanates (R'-N=C=O) and cyanates (R'-O-C≡N)

- Isothiocyanates:
  isothiocyanates: R'-N=C=S

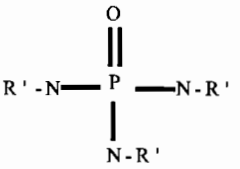
- Carbodiimides:
  carbodiimides: R'-N=C=N-R'

- Organic nitrates and nitrites:
  organic nitrates (R'-O-NO_2) and organic nitrites R'-O-NO

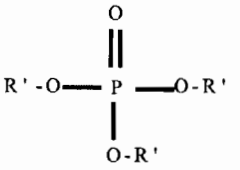
Phosphorus Functional Groups

Chemicals containing phosphorus functional groups include phosphoramides; phosphates (-PO_4) and phosphites (-PO_3); and phosphonate esters and phosphonium functional groups. Chemicals containing both sulfur and phosphate functional groups include the mono- and dithio-phosphate esters.

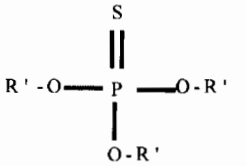
- Phosphoramides

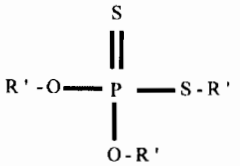


- Phosphates and thiophosphates:
  phosphates (*center*)

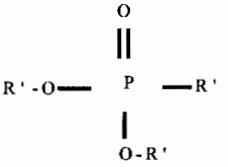


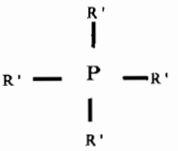
thiophosphates (*center*) and dithiophosphates (*right*)





- Phosphonate esters and phosphonium salts:
  phosphonate esters (center) and phosphonium ion (right)





Sulfur Functional Groups

Chemicals which contain sulfur functional groups include: thioamides; substituted thioureas; thioethers; sulfamates; sulfate (-SO_4) and sulfite (-SO_3) esters; sulfonate and sulfinyl esters; and dithiols and aromatic thiols. Chemicals containing both sulfur and nitrogen functional groups include thiocarbamates and isothiocyanates. In contrast, heterocyclic chemicals with sulfur atoms in the ring structure are included in the section entitled "**Sulfur Heterocyclic Chemicals**" along with other heterocyclic chemicals.

- Thiocarbamic acids:
  thiocarbamic acid esters: R'-NH-C(S)-OR'

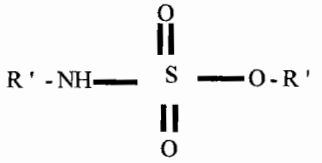
- Isothiocyanates:
  isothiocyanates: R'-N=C=S

- Thioamides:
  thioamides: R'-CS-NH-R'

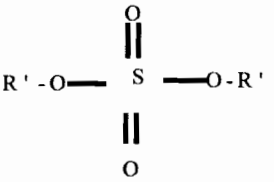
- Substituted thioureas:
  substituted thioureas: R'-NH-CS-NH-R'

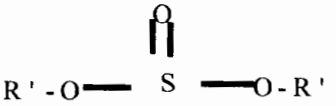
- Thioethers: Group includes thioethers, disulfides and trisulfides
  e.g. thioethers: R'-S-R'

- Sulfamates:



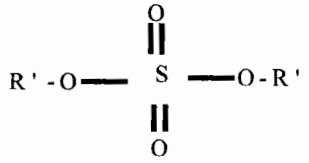
- Sulfate and sulfite esters:
  sulfate ester (center) and sulfite esters (right)

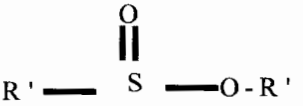


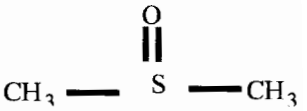


- Sulfonate esters, sulfinyl esters and sulfoxides:

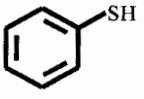
  sulfonate esters (left), sulfinyl esters (center) sulfoxides (right, e.g. dimethyl sulfoxide) and sulfones.







- Aromatic thiols and dithiols: Group includes both aromatic (and other cyclic) thiols and dithiols (cyclic and non-cyclic).
  aromatic thiols (*e.g.* benzenethiol)



dithiols (*e.g.* 1,2-propanedithiol: CH_3-CH(SH)-CH_2-SH)

Heterocyclic Chemical Structure Categories

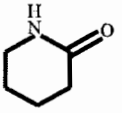
Heterocyclic chemicals include chemicals that contain within the ring structure a nitrogen, oxygen, or sulfur atom. In addition, some heterocyclic chemicals contain ring structures with both nitrogen and oxygen atoms; nitrogen and sulfur atoms; oxygen and sulfur atoms; and all three, nitrogen, oxygen and sulfur atoms.

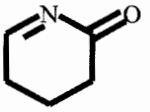
Nitrogen Heterocyclic Chemicals

Heterocyclic chemicals containing a nitrogen atom within the aromatic ring include: acridines; aziridines; carbazoles; imidazoles, triazoles, and benzotriazoles; indoles; lactams; piperidines; pteridines; purines; pyrazoles and pyrazolones; pyridines and pyrazines; pyrimidines and pyrimidinetriones; pyrroles; pyrrolidines; quinolines, isoquinolines and benzoquinolines; and triazines and benzotriazines.

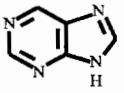
- Aziridines:

- Lactams: Category includes lactams (center) and lactims (right)

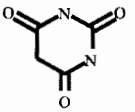




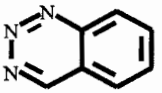
- Purines:



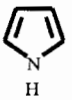
- Pyrimidines, pyrimidinetriones, triazines and benzotriazines:
  pyrimidines (center) and pyrimidinetriones (right)



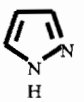
  benzotriazines (center) and triazines (right)

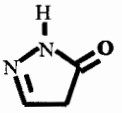


- Pyrroles:

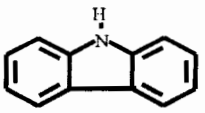


- Pyrazoles and pyrazolones:
  pyrazoles (center) and pyrazolones (right)

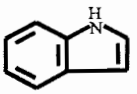




- arbazoles:

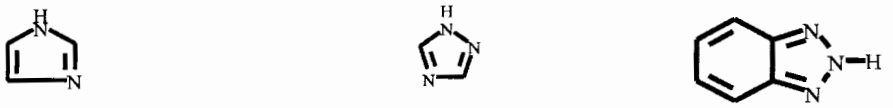


- Indoles:

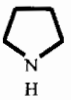


- Imidazoles, triazoles and benzotriazoles:

imidazoles (left), triazoles (center) and benzotriazoles (right)



- Pyrrolidines



- Additional nitrogen heterocyclic chemicals [*e.g.* acridines, alloxazines, benzoquinolines, naphthyridines, phthalimides, piperazines, piperidines, and pteridines, pyrazines, pyridines,

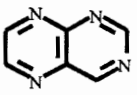
acridines (*e.g.* acridine, center) and naphthyridines (*e.g.* naphthyridine, right)

alloxazines (*center*) and phthalimides (right)

piperidines (center) and piperazines (right)

pyridines (center) and pyrazines (right)

pteridines



benzoquinolines (center) and quinolines (right)

Oxygen Heterocyclic Chemicals

The heterocyclic chemicals containing an oxygen atom within the ring

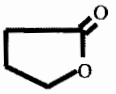
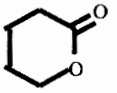
structure include: alkene/phenoxy chemicals; dioxanes; epoxides; furans and benzofurans; oxetanes; pyrans and benzopyrans; saturated lactones, and α-,β-unsaturated lactones. In addition, certain oxygen substituted heterocyclic chemicals have been included in this section, including: anthraquinones, benzoquinones, quinones, and thioxanthones.

- Epoxides: Group contains three membered mono- and poly-functional epoxides. This category also contains peroxides which are not heterocyclic.

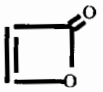
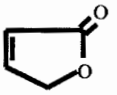
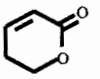
mono-epoxides (*e.g.* ethylene oxide)

peroxides (*e.g.* hydrogen peroxide: H_2O_2)

- Saturated lactones:

- α,β-Unsaturated lactones:

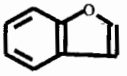
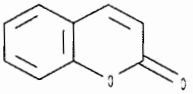




- Dioxanes:

e.g. 1,4-dioxane

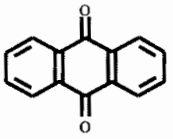
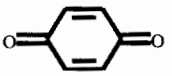
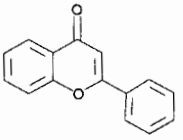
- Furans, benzofurans and coumarins:

furans (left), benzofurans (center), and coumarins (*right*)

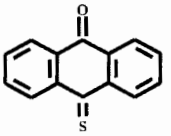




- Anthraquinones, benzoquinones, flavones, pyrones and thioxanthones:

e.g. anthraquinone (left), benzoquinone (center), and flavones (right)

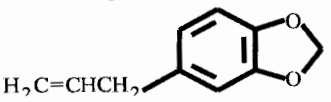




e.g. pyrone (center) and thioxanthone (right)

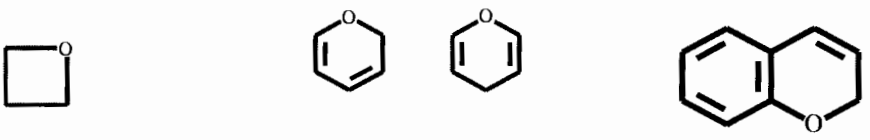
- Aromatic ethers with alkene functional groups: Group contains chemicals with safrole-like structures (*i.e.* mono-aromatic ethers with a conjugated alkene functional group).

e.g. safrole



- Oxygen heterocyclic chemicals [*e.g.* oxetanes, pyrans, and benzopyrans]:

oxetanes (left), pyrans (center) and benzopyrans (right)



Sulfur Heterocyclic Chemicals

- Heterocyclic chemicals containing sulfur [*e.g.* sulfones, trithianes, thienes, thiones, and thiophenes]:

sulfones (center) and trithianes (right)

thiones (center) and thiophene (right)

Nitrogen and Oxygen Heterocyclic Chemicals

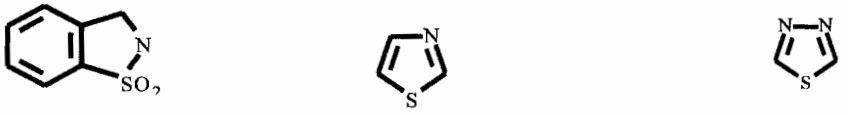
- Heterocyclic chemicals containing nitrogen and oxygen [*e.g.* morpholines and oxazoles]:

morpholines (center) oxazoles (right)

Nitrogen and Sulfur Heterocyclic Chemicals

- Heterocyclic chemicals containing nitrogen and sulfur [*e.g.* sulfimides, thiadiazoles, thiazides, thiazines and thiazoles]:

sulfimides (left), thiazoles (center) and thiadiazoles (right)

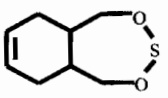


thiazides (center) and thiazines (*e.g.*, phenothiazine, right)

Oxygen and Sulfur Heterocyclic Chemicals

- Heterocyclic chemicals containing oxygen and sulfur [*e.g.* oxythiepins]:

oxythiepins (*e.g.* 2,4,3,-benzodioxathiepin)



Nitrogen, Oxygen and Sulfur Heterocyclic Chemicals

- Heterocyclic chemicals containing nitrogen, oxygen and sulfur [*e.g.* oxythiazins]:

Inorganic and Organometallic Chemicals

- Inorganic salts: Group contains inorganic salts that are not included in Categories **A** and **B** (*e.g.* aluminum). It also contains non-covalent complexes of these inorganic chemicals with organic chemical

  e.g. aluminum ammonium sulfate: $AlNH_4(SO_4)_2$

- Organometallic chemicals:
  e.g. vitamin B_{12}

Mixtures

- Mixtures: Group contains compounds that are mixtures of chemicals, and two types of mixtures are distinguished. The first type of mixture includes defined mixtures containing one or more substances which possess functional groups listed in Category **C**. The second type of mixture includes mixtures of undefined composition, including compounds in which all known components contain functional groups listed in Categories **A** and **B**. This conservative approach was taken, because it is possible that a minor, undefined constituent of a compound mixture could have a chemical with a functional group listed in Category **C**.

III B 3. Estimation of Human Exposure to Direct Food Additives and Food Ingredients

A key factor in the safety evaluation of a food additive or food ingredient is the relationship of its probable human exposure to the level at which adverse effects are observed in animal and/or clinical studies. Estimates of probable human exposures require knowledge of the specific uses and use levels of a substance under consideration and quantitative information on intakes of the foods in which the substance is used. Individuals' food intakes are distributed over a range determined by lifestyles and localized patterns of food availability and can be expected to change in response to changes in economic circumstances, education, health, the media, and the availability of products in the food supply [30] Because of the many factors affecting food intakes and the uncertainties in the eventual marketing of a petitioned food ingredient/additive, the estimation of probable exposures is a complex exercise. The Agency's assumptions concerning intake patterns, market penetration, and substance concentrations result in a conservative estimate of exposure. (When comparing estimated daily intakes (EDIs), a more conservative EDI is higher.) These assumptions are used because detailed information that can replace these assumptions is usually unavailable.

CFSAN's estimates of probable human exposure are based on food intake or food availability data obtained over relatively short time frames (one day to one year) and are used to represent chronic or "lifetime" exposure. We typically use the 90th percentile to represent probable exposure for a "heavy" consumer of a substance.

a. Parameters for the Exposure Estimate

In the broadest sense, two factors are required for making an estimate of exposure to a substance in the food supply. The first is the daily intake of the food in which the substance is used or can be found. The second is the concentration or use level of the substance in the food. Simple multiplication of these two factors gives an estimate of exposure to the substance from consumption of the food.

These two factors can be derived from a number of sources. For pre-market approval of new substances, information on the expected use level (or in the case of processing aids, expected residue concentration) in food is generally supplied by the petitioner. For substances already in the food supply, for which a cumulative exposure estimate incorporating proposed new uses is needed, use levels in food may be obtained from additional sources, such as Agency records, users of the substance, or by chemical analyses of the foods in which the substance is known to be used.

The daily intake of foods can be derived from a variety of data bases. The three most commonly used sources of food-intake data are: per-capita data derived from annual poundage surveys of producers or distributors; survey data on the frequency of consumption of foods ("food-frequency" surveys); and food-intake survey data. These three data base types will be described in more detail below. A number of data bases currently available for determining food intakes for estimating exposure to substances in the diet are shown in **Table 1** below.

b. Estimates of Food Intake

i. Annual Poundage Information (Disappearance Data)

Information on the poundages of commodities entering commerce is usually available from government and industry sources on an annual basis. These data are referred to as "disappearance" data. It is generally not possible to separate out the fraction actually consumed as food from that remaining in inventory and from non-food expenditures from inventory (wasted, exported, used in pet food or animal feed, etc.) at the end of the reporting period. Annual disappearance figures can be divided by the national population and by 365 days to obtain a "per capita" daily intake of the commodity.

Annual poundages of some substances produced and used solely for addition to food have been compiled as a part of the National Academy of Sciences Survey of Industry on the Use of Food Additives (National Research Council, 1977, 1982, and 1987). Industry' responses to these surveys are voluntary, and the reliability of these data depends heavily on the completeness of the industry response for a given substance. In order to correct for under-reporting in such surveys, a correction factor is generally employed. This factor is related to the percentage of users of a substance that submitted information to the survey.

Table 1

Summary of Characteristics of Major Databases Used for Estimating Intake of Substances

	NFCS[1]	NFCS Foods Commonly Consumed[2]	NFCS CSFII[3]	NHANES II[4]	MRCA Menu Census[5]	NPD National Eating Trends[6]	Seafood Consumption[7]	USDA ERS[8]	FDA Total Diet Study[9]
Date of data collection	1977-78[10] 1987-88	1977-78	annual since 1985	1976-80	every 5 years since 1957	annual	1973-74	annual since 1909	annual since 1961
Population surveyed	civilian; free-living; all ages	see NFCS	core & poverty groups of women 19-50 yr & their children 1-5 yr	civilian; free-living; 6 mo to 74 yr	participants in consumer survey; all ages	participants in consumer survey; all ages, 11 age-sex groups reported	participants in consumer survey; all ages, single person households excluded	net commodities in US commerce	since 1982 nationally representative diets for 8 age/sex groups
Type of instrument for intake	1-d recall & 2-d record	see NFCS	self; proxy for child	self (private interview); proxy for child	homemaker reports for all	one respondent for household	one respondent for household	NA	NA
Estimates possible	single day; usual	eaters only intake/d (mean and percentiles)	single day; usual	single day	single day; usual	single day; usual	single day; usual	per capita availability	usual
Form of Estimate	mean frequency & distribution for raw data total population and eaters only	mean and distribution of eaters	mean frequency & distribution for raw data total population and eaters only	mean frequency & distribution for raw data total population and eaters only	mean frequency & distribution for raw data total population and eaters only	mean & distribution for total population and eaters only	mean number of servings; % of individuals using product	population mean	since 1982, mean + sd

See next page for footnotes.

Footnotes for Table 1

1. U.S. Department of Agriculture. Nutrient Intakes: individuals in 48 states, year 1977-78. Nationwide Food Consumption Survey (NFCS) Report No. 1-2. Available from: U.S. Government Printing Office, Washington DC.

2. Pao, E.M., Fleming, K.H., Guenther, P.M., and Mickle, S.J. (1982) Foods Commonly Eaten by Individuals: Amount per Day and per Eating Occasion. Home Economics Research Report No. 44. Available from the U.S. Government Printing Office, Washington, DC.

3. U.S. Department of Agriculture (1985) Nationwide Food Consumption Survey: Continuing Survey of Intake by Individuals, women 19-50 years and their children 1-5 years, 1 day. CSFII Report No. 85-1. Available from the U.S. Government Printing Office, Washington, DC.

4. McDowell, A.D., Engel, A., Massey, J.T., and Maurer, K. (1981) Plan and Operation of the Second National Health and Nutrition Examination Survey, 1976-1980. Vital and Health Statistics. Series 1, No. 15. DHHS Publication No. (PHS)81-1317. Available from the U.S. Government Printing Office, Washington, DC.

5. Abrams, I.J. (1981) Mail diary method for collecting food purchasing and food usage information from consumer panels. In, Assessing changing food consumption patterns. Washington, DC: National Academy Press, pp. 119-134.

6. The NPD Group of Marketing and Research Services (1988) National Eating Trends. A research study on in-home food and beverage consumption. Available from The NPD Group of Marketing and Research Services, Park Ridge, IL.

7. Hu, T.-W. (1985) Analysis of Seafood Consumption in the US: 1970, 1974, 1978, 1981. Report prepared for the National Marine Fisheries Service, Washington, DC, under grant no. NA82AA-H-00053 by the Institute for Policy Research and Evaluation, the Pennsylvania State University, University Park, PA. p. 94. Available from The National Marine Fisheries Service, Washington, DC.

8. Bunch, K.L. (1985) Food consumption, prices, and expenditures 1985. Statistical Bulletin No. 749. Available from: U.S. Government Printing Office, Washington, DC.

9. Pennington, J.A.T. and Gunderson, E.L. (1987) A history of the Food and Drug Administration's Total Diet Study, 1961-1987. J.Assoc. Off.Anal.Chem. 70:772-782.

10. This survey has been criticized in a General Accounting Office Report entitled "NUTRITION MONITORING Mismanagement of Nutrition Survey Has Resulted in Questionable Data", GAO/RCED-91-117, Washington DC. Care should be exercised before employing data taken from this survey.

i. Dietary Surveys of Food Intake

Food-intake surveys provide data that are commonly used to estimate exposure to a food additive or ingredient. Two different types of surveys exist: daily-consumption surveys and "food-frequency" surveys, i.e; surveys of the frequency of consumption or number of eating occasions of a given food on a given day. Daily-consumption surveys require participants to record or recall the amounts and types of each food eaten during the day. Food-frequency surveys require participants to record only the number of times each food is consumed during each day of the survey period; these frequencies need to be multiplied by a portion size to obtain the daily food-intake information.

These survey methods have the advantage of providing several different kinds of information about food consumption. That is, food intakes of various sub-groups (*e.g.* sex, age, eaters-only, total sample *etc.*) can be obtained for either the total diet or for specific foods. Eaters-only intake data are useful for determining intake of a food used by a small percentage of the population and by individuals selecting for a particular product. In such cases, use of the information derived from the responses of the total sample will generally yield intake figures that are much lower than actual intake. Daily-consumption surveys and food-frequency surveys can also provide valuable information for short-term intake (*i.e.* days to years).

Food-intake survey data are essential when information is required concerning the potential for very high use among consumers. Because these data are compiled from information obtained from individual consumers, it is possible to determine a distribution of intakes. The 90th percentile intake estimate (that intake which is equal to or higher than 90 percent of the intakes for all individuals surveyed) is used to represent the intake of heavy consumers of a substance.

For a substance not expected to be consumed frequently, the use of surveys of short duration (1-3 days) often leads to an overestimate of chronic intake of the food containing the substance. This is due to a variety of circumstances beyond the scope of this discussion (see the FASEB report noted previously on page 43). Additionally, the use of longer-term surveys (*e.g.* 14 days) is generally preferred for estimating exposure that is more likely to reflect chronic intake.

iii. Substance Concentration Data (Petitioner-Supplied Data)

When seeking pre-market approval or approval for a new use of a regulated substance, the petitioner is required to supply information concerning the intended use levels of the substance in food. This information is often supplied as a maximum use level. If demonstrable, a technologically self-limiting concentration can be supplied and used in the estimation of probable intake. Usually, the petitioner will supply a "typical" or "recommended" use level, based on in-house experimentation, which can be used in the exposure estimation process. The Agency can determine which type of information is most pertinent on a case-by-case basis, usually using the information that yields a conservative, yet reasonable exposure estimate (see previous discussion, Chapter II C).

c. Preparing Exposure Estimates

i. Estimating Exposure for Pre-Market Evaluations

Pre-market estimations are intended to represent conservative yet reasonable estimates of exposure to a new substance used in food. Information concerning potential use levels is supplied by the petitioner. Food-intake data are obtained by Agency reviewers from the above mentioned data bases and other appropriate sources, including the petitioner. One basic assumption for making an exposure estimate is that all food ingested by a consumer that may contain the additive or ingredient, does contain it at the recommended or maximum level of use.

A major issue in the pre-market estimation of exposure is the choice of the data base used to determine representative food intake. While broad generalizations can be made, each case requires an examination of the suitability of various data bases with respect to the availability of, or necessity for, information concerning age, sex or other sub-groupings, the extent of consumer awareness of the substance, and the potential ubiquity of the substance in the diet.

a) Per-capita Estimates

In the absence of food intake data, per-capita disappearance data for

commodities may be used to make a pre-market exposure estimate for a food additive or ingredient that is expected to have no market appeal (one that will not be sought out by consumers) or that is expected to be ubiquitous in the diet. For these purposes, "per-capita" generally refers to the number of people in the United States. For example, the annual poundage of the commodity first is converted to grams per day (the calculation can be modified if information about non-food uses of the commodity is known). This daily intake figure is then multiplied by concentration of the substance in the commodity (based on the intended use) to yield the probable exposure. One example of this procedure would be the estimation of exposure to an anti-dust spray used in grain silos. The petitioner would have determined the optimal amount of substance sprayed onto a given amount of grain. This concentration would be multiplied by the daily per-capita disappearance of the grain to determine the per-capita exposure to the anti-dust agent. Other information could be factored into this calculation, if available; for example, information about the loss of treated grain during storage and the effects of processing on the quantity of the anti-dust agent that would ultimately be ingested by a consumer.

Conservatism in a per-capita estimate arises from the inability to determine how much of the commodity is lost in storage, waste, or processing, remains in inventory, is exported, or is used in non-food applications. This type of estimate cannot directly produce an upper percentile intake estimate for a substance (see discussion below for making such estimates).

Per-capita estimates of exposures to substances that have been surveyed by the NAS (food use only) can be made by dividing the reported annual poundages by the current population and converting to daily usage. Conservatism in this type of estimate again arises from the inability to separate wastage, inventory, and loss of the substance in processing from the total actually consumed via the food supply.

Per-capita estimates usually are inappropriate in cases where the use of the additive or ingredient is highly limited, when only a limited number of consumers are eaters of foods containing the added substance, or when a consumer can select for the substance in food.

b) Survey-based Estimates

Because dietary intake or food-frequency surveys contain the most detailed information about the subjects' eating habits, they are the preferred source of food-consumption data for use in estimating exposure. In the simplest case, the average daily intake or the intake for a given percentile of a food containing the substance of interest is multiplied by the concentration of the substance in that food to yield the exposure estimate. For a substance expected to be used in several foods the problem is more complex. The Agency currently has access to food-intake information that is aggregated across groups of individuals and food categories, (*i.e.* data bases containing consumption information based on general food categories and sub-categories, for example, baked goods, milk and milk products). Using the aggregated food-intake data, the Agency can estimate the mean total exposure (*i.e.* total sample basis) to a petitioned substance by adding mean exposures to the substance calculated for the individual food categories.

When considering eaters-only intakes, additional considerations should be included. Simple addition of mean, eaters-only intakes may lead to an exaggeration of the mean intake. For example, if a substance is to be used in both regular and diet soft drinks, an overestimation of intake is likely to result from addition of the potential exposures to the substance from each type of soft drink. Consumers usually drink one or the other of these beverages, but not both. Typically, the higher exposure estimate (for, in this case, diet or regular soft drinks) would be used in place of the summed value. The same would be true for potential exposure to an additive from different types of snack foods, such as pretzels and potato chips. Therefore, in calculating the mean eaters-only exposure to additives, caution must be exercised in determining whether exposures derived from aggregated food categories should be added.

A specific percentage intake of an additive that may be used in different food groups can be estimated using different methodologies. We have noted that 90th percentile intakes are typically 2 to 3 times the mean intake. The intake of a heavy consumer of an additive can be approximated by multiplying the derived mean intake by a factor of two to three. (Survey intake data from the individual foods provide 90^{th} percentile/mean ratios, which can be averaged to determine the factor used.) Also, computer-based modeling, such as a Monte Carlo simulation, can be used to statistically derive distributions of intake for a total sample or eaters-only population. Monte Carlo modeling methods have been described in the literature. [31-35]

An alternate approach for estimating eaters-only intakes involves the estimation of exposure derived from dietary survey analyses based on the food consumption of each surveyed individual. Such exposure estimates can be made only by accessing the raw data from the survey. Although the Agency does not have ready

access to such data, petitioners have in some instances contracted with owners of raw survey data to provide exposure estimates based on specific information about food uses and use levels of petitioned additives or ingredients. The Agency uses its judgement in considering the manner in which intake estimates for individual food categories should be combined to estimate total exposure. The ability to manipulate the raw survey data permits the actual intake of each food for each surveyed individual to be combined with the proposed use level for the additive or ingredient in the specific foods eaten by that individual. This allows the construction of a distribution curve based on total additive or ingredient intake for each individual; from this curve the desired percentile information on exposure may be obtained. Given that the conservatism inherent in the use of aggregate data has been removed, exposure analyses based on intakes of individuals that have been submitted by a petitioner are carefully evaluated for their appropriateness for predicting probable chronic intake of the substance.

Finally, special cases may arise, particularly in the area of substances that could become macro-ingredients in the diet, for which food-consumption and use-level information necessary for estimation of exposure are inadequate or unavailable. For example, difficulties can arise in estimating intake when current eating habits cannot be reliably extrapolated to include the new substance. In such cases, new approaches to the pre-market estimate will have to be devised. The use of substitutes for added fats illustrates this point. In this example, the diet as a whole, especially the amount of energy needed to maintain normal function, needs to be considered if such a substitute would be marketed to consumers with no restrictions on its use.

ii. Exposure Estimates for Substances Currently in the Market Place

Updated exposure estimates are needed for substances on the market when a new use of an approved substance is petitioned or when intake is believed to have changed appreciably from the time of the original estimate. The approaches available for making this type of estimate are similar to those for pre-market approvals, with the advantage that more information is generally available on the substance, including, but not limited to, actual levels in foods.

Detailed intake estimates can be made using dietary survey information and actual substance use levels. For a new use of an existing substance, a cumulative estimate can be made by combining the appropriate use level and food-intake data for the new use, and adding this estimate to the more accurate estimate available for the existing uses. Alternatively, new data based on an analysis of intakes by individuals and covering both regulated and proposed uses may be submitted by the petitioner. As discussed above for pre-market approvals, estimates for desired sub-groups (age, sex, 90th percentile eaters, eaters-only) can be obtained using these dietary survey data.

d. Conversion Factors

Exposure estimates are commonly presented in grams per person per day (g/p/d), milligrams per kilogram body weight (mg/kg bw), or parts per million of the daily diet. To convert among these unit types, we typically use the following factors: a 60 kg "typical adult," and a total daily diet of 3000 g food and water (1500 g solid food, 1500 g liquid food). For those cases where information concerning children is needed, we have used a body weight of 15 kg for a 2-5 year old child.

e. Summary

The Federal Food, Drug, and Cosmetic Act (sec. 409 (c)(5)(A)) requires that probable consumption of an additive and of any substance formed in or on food because of its use be considered in determining whether the proposed use is safe. FDA's estimates of probable consumption are generally made using existing commodity disappearance data and food-intake and food-frequency data bases, occasionally supplemented with ad hoc approaches and reasoned judgments. Reasonable exposure estimates for chemicals used in food are critical to the maintenance of a safe food supply. Additional information concerning the preparation of estimates of exposure to food additives is available from the FDA's Center for Food Safety and Applied Nutrition.[36]

III C. Recommended Toxicity Tests

1. Recommended Minimum Set of Toxicity Tests

The extent and type of toxicity testing recommended for direct food additives or color additives used in food will depend on the initial Concern Level

to which that additive has been assigned and available information about the metabolism, chemical composition, and toxicity of the additive. Recommendations for minimum testing are associated with each Concern Level, and these recommendations reflect the Agency's consensus that extensive toxicity testing should be reserved for additives with high exposures and potentially reactive structures and for additives that induce adverse toxic effects at low doses or after short exposures (see **Table 2** below).

The final extent and type of toxicity testing recommended for a food or color additive will be determined by estimated exposure and potential toxic effects (dose, onset, duration, type, extent, *etc.*) observed in the minimum set of tests recommended for the additive.

a. Minimum Set of Toxicity Tests for Concern Level III Substances

The recommended tests for Concern Level III substances are sensitive enough to detect nearly all types of observable toxicity, including malignant and benign tumors, pre-neoplastic lesions, and most other signs of chronic toxicity. They include:

- short-term tests for genetic toxicity;
- metabolism and pharmacokinetic studies;
- a subchronic feeding study (at least 90 days in duration) in a rodent species, which includes an evaluation of the potential neurotoxicity and immunotoxicity of the test substance;
- a multi-generation reproduction study (two generations, one litter per generation) with a teratology phase (developmental toxicity study) in a rodent, which includes an evaluation of the potential developmental neurotoxicity and immunotoxicity of the test substance;
- a long-term (at least one year in duration) feeding study in a non-rodent species; and
- carcinogenicity studies on two rodent species. At least one of these studies should be a combined chronic toxicity/carcinogenicity study with an *in utero* exposure phase.

The results of short-term tests for genetic toxicity may be used to determine priority for the conduct of lifetime carcinogenicity bioassays, and may assist in evaluating the results of bioassays. Results of metabolism and pharmacokinetic studies can be used to help set appropriate dose levels in toxicity studies and evaluate the results of those studies; information from metabolism and pharmacokinetic studies also may be used to modify the set of toxicity studies recommended for a particular additive (for example, concern about an additive may be reduced if the additive is shown to be largely unabsorbed by humans). Results from the reproduction study with teratology phase may indicate the need for expanded reproduction and/or developmental toxicity tests. Results of immunotoxicity and neurotoxicity screens in subchronic studies and developmental toxicity studies may indicate the need for further testing in these areas.

b. Minimum Set of Toxicity Tests for Concern Level II Substances

The tests recommended for Concern Level II substances are sensitive enough to detect most toxic phenomena other than late-developing histopathological changes in tissues and organs. Tests recommended for food and color additives used in food assigned to Concern Level II are:

- short-term tests for genetic toxicity;
- metabolism and pharmacokinetic studies;
- a subchronic feeding study (at least 90 days in duration) in a rodent species, which includes an evaluation of the potential neurotoxicity and immunotoxicity of the test substance;
- a subchronic feeding study (at least 90 days in duration) in a non-rodent species, which includes an evaluation of the potential neurotoxicity and immunotoxicity of the test substance; and
- a multi-generation reproduction study (two generations, one litter per generation) with a teratology phase (developmental toxicity study) in a rodent. This study includes an evaluation of the potential developmental neurotoxicity and immunotoxicity of the test substance.

c. Minimum Set of Toxicity Tests for Concern Level I Substances

Recommended tests for Concern Level I substances include:

- short-term tests for genetic toxicity and
- a short-term feeding study (at least 28 days duration) in a rodent species, which includes an evaluation of the potential neurotoxicity and immunotoxicity of the test substance.

The results of short-term tests for genetic toxicity may suggest the need for information about the additive that can be obtained from chronic toxicity or carcinogenicity tests. The short-term feeding study is sensitive enough to detect any acute, life-threatening toxicity and to provide an indication of target organs and doses for toxicity tests of longer duration, if such tests are recommended. Results of immunotoxicity and neurotoxicity screens in the short-term feeding study may indicate the need for further testing in these areas.

Table 2

Summary of the Toxicity Tests Recommended for Different Levels of Concern

Toxicity Studies [1]	Concern Levels		
	I	II	III
Short-term Tests for Genetic Toxicity	X	X	X
Metabolism and Pharmacokinetic Studies		X	X
Short-term Toxicity Studies with Rodents	X [2]		
Subchronic Toxicity Studies with Rodents		X [2]	X [2]
Subchronic Toxicity Studies with Non-Rodents		X [2]	
Reproduction Studies with Teratology Phase		X [2]	X [2]
One-year Toxicity Studies with Non-Rodents			X
Carcinogenicity Studies with Rodents			X [3]
Chronic Toxicity/Carcinogenicity Studies with Rodents			X [3,4]

[1] Not including dose range-finding studies, if appropriate
[2] Including neurotoxicity and immunotoxicity screens
[3] An *in utero* phase is recommended for one of the two recommended carcinogenicity studies with rodents, preferably the study with rats
[4] Combined study may be performed as separate studies

III C 2. Selecting Additional Toxicity Tests

Deciding how much information is sufficient to assess the safety of an additive is a problem that has long been recognized both by the Agency and industry. Results from the initial set of recommended toxicity tests for direct food additives and color additives used in food may indicate a need for additional or specialized testing to assess the safety of the additive. Additional recommended tests will depend, in large part, on effects observed in the initial set of recommended toxicity tests. The purpose of this section is to provide examples of how FDA decides what additional toxicological information needs to be developed for a direct food additive or color additive used in food, based on evaluation of data obtained from studies submitted by the petitioner in support of the safety of an additive. The examples are not intended to be comprehensive. Decisions about the need for additional toxicology information on food and color additives used in food will be made on a case-by-case basis, will always include a significant element of expert scientific judgement, and thus may differ from examples presented below.

a. Acute Toxicity Tests

Acute toxicity tests (usually single-dose tests in which animals are observed for 7-14 days following administration of the test substance) may be recommended for compounds when there is no other information that can be used to select appropriate dose levels for short-term or subchronic toxicity tests.

b. Short-Term Toxicity Tests with Rodents and Non-Rodents

Short-term feeding tests with rodents or non-rodents (usually studies in which animals are exposed to continuous oral doses of the test substance for one month or less) may be recommended for compounds when there is no other information that can be used to select appropriate dose levels for subchronic or chronic studies.

c. Subchronic Toxicity Tests with Rodents

Subchronic toxicity tests (usually studies in which animals are exposed to continuous oral doses of the test substance for 90 days to 12 months) may be recommended for Concern Level I compounds with a lowest observed effect level (LOEL) from a shorter-term study which is less than 2000 times the estimated human consumption of the compound.

Subchronic toxicity studies may be recommended for compounds when there is no other information that can be used to select appropriate dose levels for longer-term toxicity studies.

d. One-Year Toxicity Tests with Non-Rodents

One-year toxicity tests in non-rodents may be recommended for Concern Level II compounds when the lowest observed effect level (LOEL) from a shorter-term, non-rodent study is less than 1000 times the estimated human consumption of the compound, particularly if the non-rodent species is the species most sensitive to the effect and is appropriate for extrapolation to man.

One-year toxicity tests in non-rodents may be recommended for Concern Level II compounds when available toxicology information suggests the probability that the compound bioaccumulates and/or is associated with late-occurring toxicity in rodents; such late-occurring toxicity may not be observed or may be poorly quantified in subchronic studies.

e. Carcinogenicity Studies with Rodents

Carcinogenicity bioassays in two rodent species may be recommended for Concern Level I and II compounds when data from other studies indicate treatment-related hyperplasia, metaplasia, or other proliferative lesions, or when data from other studies indicate progressive and irreversible lesions, such as treatment-related necrosis. Carcinogenicity bioassays also may be recommended for Concern Level I or II compounds that have demonstrated significant carcinogenic potential, based on the results of short-term tests for genetic toxicity.

f. Two-Generation Reproduction Studies with a Teratology Phase

Two-generation reproduction studies with a teratology phase may be recommended for Concern Level I compounds when results from other toxicity studies indicate that the compound may be associated with reproductive organ toxicity.

Two-generation reproduction studies with a teratology phase may be recommended for Concern Level I compounds that have demonstrated significant carcinogenic potential, based on the results of short-term tests for genetic toxicity.

g. Gavage Administration of the Test Compound in Teratology Studies

Gavage administration of the test compound in teratology studies may be recommended when the estimated human exposure exceeds 0.625 mg/kg/day in the diet.

Gavage administration of Concern Level III test compounds in teratology studies may be recommended when the compound is expected to be added to beverages that may be consumed by pregnant women.

Gavage administration may be recommended for compounds with adverse reproductive effects that suggest possible teratogenicity.

h. Metabolism and Pharmacokinetic Studies

Additional metabolism and pharmacokinetic studies may be recommended for any compound when results of the recommended set of studies do not resolve important metabolic information, such as whether or not the food additive is absorbed in significant amounts from the gastrointestinal tract.

i. Neurotoxicity Studies

Neurotoxicity studies may be recommended for any compound when results from the neurotoxicity screen or other information suggests that the compound may be associated with neurotoxic effects.

j. Immunotoxicity Studies

Immunotoxicity studies may be recommended for any compound when results from the immunotoxicity screen or other information suggests that the compound may be associated with immune system toxicity.

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