

Establishing the Relevance of Health Hazard Data for GHS Classification: Adverse vs. Non-Adverse

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Society for Chemical Hazard Communication

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Health Hazard Evaluation and HazCom

 The appropriate health hazard classification of substances and mixtures* is the foundation of an effective, informative and scientifically defensible hazard communication program.

* This presentation will use "chemical" or "chemicals" for simplicity

Health Hazard Evaluation and GHS

1.3.2.1.2 One objective of the GHS is for it to be simple and transparent with a clear distinction between classes and categories in order to allow for "self-classification" as far as possible. For many hazard classes the criteria are semi-quantitative or qualitative and expert judgement is required to interpret the data for classification purposes. Furthermore, for some hazard classes (e.g. eye irritation, explosives or self-reactive substances) a decision tree approach is provided to enhance ease of use.



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Health Hazard Evaluation and GHS

1.1.2.6.2 Hazard vs. risk

1.1.2.6.2.1 Each hazard classification and communication system (workplace, consumer, transport) begins coverage with an assessment of the hazards posed by the chemical involved. The degree of its capacity to harm depends on its intrinsic properties, i.e. its capacity to interfere with normal biological processes, and its capacity to burn, explode, corrode, etc. This is based primarily on a review of the scientific studies available. The concept of risk or the likelihood of harm occurring, and subsequently communication of that information, is introduced when exposure is considered in conjunction with the data regarding potential hazards. The basic approach to risk assessment is characterized by the simple formula:

hazard \times exposure = risk

Hazard \neq Risk

Hazard = Intrinsic ability to damage biological material

Risk = Probability (potential) for the hazard(s) to be expressed in a given situation/scenario

Safety is the inverse of risk (Safety = 1/Risk; \uparrow Risk = \downarrow Safety)

GHS and [Health] Hazard Classification

1.3.2.2 Concept of "classification"

1.3.2.2.1 The GHS uses the term "hazard classification" to indicate that only the intrinsic hazardous properties of substances or mixtures are considered.

- 1.3.2.2.2 Hazard classification incorporates only three steps, i.e.:
 - (a) identification of relevant data regarding the hazards of a substance or mixture;
 - (b) subsequent review of those data to ascertain the hazards associated with the substance or mixture; and
 - (c) a decision on whether the substance or mixture will be classified as a hazardous substance or mixture and the degree of hazard, where appropriate, by comparison of the data with agreed hazard classification criteria.

Health hazard classification of chemicals can, in some cases, be fairly straight-forward such as for acute toxicity, but become more complicated, on a relative basis, such as for STOT-SE and STOT-RE.

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GHS and [Health] Hazard Classification – Acute Toxicity

 Table 3.1.1: Acute toxicity hazard categories and acute toxicity estimate (ATE) values defining the respective categories

Exposure route	Category 1	Category 2	Category 3	Category 4	Categor	
Oral (mg/kg bodyweight) See notes (a) and (b)	5	50	300	2000	5000 See detailed	
Dermal (mg/kg bodyweight) See notes (a) and (b)	50	200	1000	2000	criteria in Note (g)	
Gases (ppmV) See notes (a), (b) and (c)	100	500	2500	20000		
Vapours (mg/l) <i>See notes (a), (b), (c), (d) and (e)</i>	0.5	2.0	10	20	See deta criteria Note (
Dusts and Mists (mg/l) See notes (a), (b), (c) and (f)	0.05	0.5	1.0	5	11010 ()	

Note:

Gases concentration are expressed in parts per million per volume (ppmV).

Notes to Table 3.1.1:

Locate "point estimate" of acute toxicity \rightarrow apply Table 3.1.1 criteria

GHS and [Health] Hazard Classification – Repeat-Exposure (Dose) Toxicity*

Results:

- body weight and body weight changes;
- food consumption, and water consumption, if applicable;
- toxic response data by sex and dose level, including signs of toxicity;
- nature, severity and duration of clinical observations (whether reversible or not);
- results of ophthalmological examination;
- sensory activity, grip strength and motor activity assessments (when available);
- haematological tests with relevant base-line values;
- clinical biochemistry tests with relevant base-line values;
- terminal body weight, organ weights and organ/body weight ratios;
- necropsy findings;
- a detailed description of all histopathological findings;
- absorption data if available;
- statistical treatment of results, where appropriate.

Many potential observations that may indicate a "target organ" effect(s). Which one(s) indicate an adverse health effect on which to base a scientifically-defensible health hazard classification?

Source: Repeated Dose 90-Day Oral Toxicity Study in Rodents (OECD No. 408)

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28. The following haematological examinations should be made at the end of the test period and when any interim blood samples may have been collected: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count and a measure of blood clotting time/potential.

29. Clinical biochemistry determinations to investigate major toxic effects in tissues and, specifically, effects on kidney and liver, should be performed on blood samples obtained from each animal just prior to or as part of the procedure for killing the animals (apart from those found moribund and/or intercurrently killed). In a similar manner to haematological investigations, interim sampling for clinical biochemical tests may be performed. Overnight fasting of the animals prior to blood sampling is recommended⁽¹⁾. Determinations in plasma or serum should include sodium, potassium, glucose, total cholesterol, urea, blood urea nitrogen, creatinine, total protein and albumin, and more than two enzymes indicative of hepatocellular effects (such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, and sorbitol dehydrogenase). Measurements of additional enzymes (of hepatic or other origin) and bile acids, which may provide useful information under certain circumstances, may also be included.

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30. Optionally, the following <u>urinalysis</u> determinations could be performed during the last week of the study using timed urine volume collection: appearance, volume, osmolality or specific gravity, pH, protein, glucose and blood/blood cells.

31. In addition, studies to investigate <u>serum markers</u> of general tissue damage should be considered. Other determinations that should be carried out if the known properties of the test substance may, or are suspected to, affect related metabolic profiles include calcium, phosphorus, fasting triglycerides, specific hormones, methaemoglobin and cholinesterase. These need to be identified for chemicals in certain classes or on a case-by-case basis.

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34. All animals in the study shall be subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The <u>liver</u>, <u>kidneys</u>, <u>adrenals</u>, <u>testes</u>, <u>epididymides</u>, <u>uterus</u>, <u>ovaries</u>, <u>thymus</u>, <u>spleen</u>, <u>brain</u> and <u>heart</u> of all animals (apart from those found moribund and/or intercurrently killed) should be trimmed of any adherent tissue, as appropriate, and their wet weight taken as soon as possible after dissection to avoid drying.

35. The following tissues should be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent <u>histopathological</u> examination: all gross lesions, <u>brain</u> (representative regions including cerebrum, cerebellum and medulla/pons), <u>spinal cord</u> (at three levels: cervical, mid-thoracic and lumbar), <u>pituitary</u>, <u>thyroid</u>, <u>parathyroid</u>, <u>thymus</u>, <u>oesophagus</u>, <u>salivary glands</u>, <u>stomach</u>, <u>small and large intestines</u> (including Peyer's patches), <u>liver</u>, <u>pancreas</u>, <u>kidneys</u>, <u>adrenals</u>, <u>spleen</u>, <u>heart</u>, <u>trachea</u> and <u>lungs</u> (preserved by inflation with fixative and then immersion), <u>aorta</u>, <u>gonads</u>, <u>uterus</u>, <u>accessory sex organs</u>, <u>female mammary gland</u>, <u>prostate</u>, <u>urinary bladder</u>, <u>gall bladder</u> (mouse), <u>lymph nodes</u> (preferably one lymph node covering the route of administration and another one distant from the route of administration to cover systemic effects), <u>peripheral nerve</u> (sciatic or tibial) preferably in close proximity to the muscle, a section of <u>bone marrow</u> (and/or a fresh bone marrow aspirate), <u>skin</u> and <u>eyes</u> (if changes were observed during ophthalmological examinations). The clinical and other findings may suggest the need to examine additional tissues. Also any organs considered likely to be target organs based on the known properties of the test substance should be preserved.

Histopathology

36. Full <u>histopathology</u> should be carried out on the preserved organs and tissues of all animals in the control and high dose groups. These examinations should be extended to animals of all other dosage groups, if treatment-related changes are observed in the high dose group.

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RE-Studies: Adverse or Non-Adverse Effect(s)?

- Repeat-exposure (dose) toxicity studies can be very complex in terms of results
 - Multiple endpoints/parameters (clinical observations, clinical chemistry, hematological, urinalysis, gross and histopathology...) that could indicate an effect(s) on which to classify under GHS
- Critical → determine whether or not the observed changes in any of these endpoint/parameters are adverse (an adverse health effect) [vs. non-adverse or adaptive]
 - Want to do [GHS] health hazard classification on treatment-related adverse health effects.

Adverse vs. Non-Adverse Effects

- Determining whether or not a health effect(s) is adverse MAY SOUND EASY but in many cases IT IS NOT.
 - A lot of times the distinction is not "obvious"
 - Or, looks obvious but really is not
 - Some frameworks for the structured evaluation of data are available
 - Guidance for the evaluation of data is also available
 - · General and specific (i.e. liver, clinical chemistries, hematology, etc.)
 - Very dependent on experience and professional judgment professional differences of opinion (interpretation of findings)
 - Can be controversial
- This determination needs to stand up to scrutiny and be scientifically defensible

Adverse Health Effect: ECHA (REACH)

 Change in morphology, physiology, growth, development or lifespan of an organism which results in impairment of its functional capacity or impairment of its capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences.

Adverse Health Effect: OECD

 A change in the morphology, physiology, growth, development, reproduction or life span of an organism, system, or (sub) population that results in an impairment of functional capacity, or an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences.

Adverse Health Effect: US EPA

 A biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism, or reduces an organism's ability to respond to an additional environmental challenge.

Adverse Health Effect: WHO/IPCS

 Change in morphology, physiology, growth, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences.

Significance*: Statistical and Biological

- <u>Statistical significance</u> (*i.e.* p < 0.05), by itself, does NOT make an effect adverse
 - May not be meaningful to the general state of health of the biological system
- If an event (effect) is not statistically significant, it may be considered adverse based on the <u>biological significance</u>
 - A response (to a stimulus) in an organism or other biological system that is considered to have substantial or noteworthy effect (positive or negative) on the well-being of the biological system.
 - Decision as to whether or not a change is biologically significant is usually left to expert judgment

Adaptive Response

- The capacity to respond to events (*e.g.* chemical exposures) in order to maintain normal function
- The process whereby a cell or organism responds to a xenobiotic so that the cell or organism will survive in the new environment that contains the xenobiotic <u>without impairment</u> <u>of function</u>*
- Common adaptive response following/during chemical exposure = liver enzyme induction

Adaptive Response

- May be completely unrelated to the inherent toxicity of the chemical
 - Liver enzyme induction → increase in the activity of [chemical] metabolizing enzymes (via increased rate of synthesis of the enzyme)
 - Liver enlargement (↑ size)
 - Increased liver weight
 - Hepatocellular hypertrophy* (↑ in size of liver paranchymal cells)
 - Elevation of serum clinical chemistry analytes (especially "liver" enzymes)

<u>Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST)</u>

* Hyperplasia is an increase in the number of cells

* Atrophy is is the diminution in size of the cell, tissue or organ

GHS and Weight of Evidence (WoE)

1.3.2.4.9 *Weight of evidence*

1.3.2.4.9.1 For some hazard classes, classification results directly when the data satisfy the criteria. For others, classification of a substance or a mixture is made on the basis of the total weight of evidence. This means that all available information bearing on the determination of toxicity is considered together, including the results of valid *in vitro* tests, relevant animal data, and human experience such as epidemiological and clinical studies and well-documented case reports and observations.

1.3.2.4.9.2 The quality and consistency of the data are important. Evaluation of substances or mixtures related to the material being classified should be included, as should site of action and mechanism or mode of action study results. Both positive and negative results are assembled together in a single weight of evidence determination.

Klimisch *et al.* A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecolotoxicological Data. *Regulatory Toxicology and Pharmacology* **25** 1-5 (1997).

Categories of data reliability:

Klimisch Code 1 = Reliable without restriction Klimisch Code 2 = Reliable with restriction Klimisch Code 3 = Not reliable Klimisch Code 4 = Not assignable

GHS and Weight of Evidence (WoE)

1.3.2.4.9.3 Positive effects which are consistent with the criteria for classification in each chapter, whether seen in humans or animals, will normally justify classification. Where evidence is available from both sources and there is a conflict between the findings, the quality and reliability of the evidence from both sources must be assessed in order to resolve the question of classification. Generally, data of good quality and reliability in humans will have precedence over other data. However, even well-designed and conducted epidemiological studies may lack sufficient numbers of subjects to detect relatively rare but still significant effects, or to assess potentially confounding factors. Positive results from well-conducted animal studies are not necessarily negated by the lack of positive human experience but require an assessment of the robustness and quality of both the human and animal data relative to the expected frequency of occurrence of effects and the impact of potentially confounding factors.

1.3.2.4.9.4 Route of exposure, mechanistic information and metabolism studies are pertinent to determining the relevance of an effect in humans. When such information raises doubt about relevance in humans, a lower classification may be warranted. When it is clear that the mechanism or mode of action is not relevant to humans, the substance or mixture should not be classified.

1.3.2.4.9.5 Both positive and negative results are assembled together in the weight of evidence determination. However, a single positive study performed according to good scientific principles and with statistically and biologically significant positive results may justify classification.

Reversibility (after cessation of exposure) and WoE: Key observation and if occurs \rightarrow may indicate a lower level of concern regarding the observed effect.

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NOAEL

<u>No-Observable-Adverse-Effect</u> Level

- The highest exposure level at which there are no statistically and biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control
 - Effects are produced at this level but are not considered to be adverse
 - Generally what we try to use as the basis for risk assessments, developing "toxicity" values (DNELs, RfDs, RfCs, etc.) and occupational exposure limits (OELs)
 - Dependent on doses used in the study

LOAEL

Lowest-Observable-Adverse-Effect Level

- The lowest exposure level that produces statistically and biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control
 - Can be used as the basis for for risk assessments, developing "toxicity" values (DNELs, RfDs, RfCs, etc.) and occupational exposure limits (OELs)
 - Dependent on the doses used in the study

NOEL



<u>No-Observable-Effect</u> Level

- The highest exposure level at which there are no statistically and no biologically significant increases in frequency or severity of effects between the exposed population and its appropriate control
 - No differences whatsoever vs. control
 - Dependent on test doses used

TABLE 4-3. EFFECT LEVELS CONSIDERED IN DERIVING INHALATION REFERENCE CONCENTRATIONS IN RELATIONSHIP TO EMPIRICAL SEVERITY RATING VALUES (Ranks are from lowest to highest severity.)^a

Effect or No-Effect Level	Rank	General Effect	
NOEL	0	No observed effects.	
NOAEL	1	Enzyme induction or other biochemical change, consistent with possible mechanism of action, with no pathologic changes and no change in organ weights.	General Guidance for NOAEL and LOAEL
NOAEL	2	Enzyme induction and subcellular proliferation or other changes in organelles, consistent with possible mechanism of action, but no other apparent effects.	Methods for Derivation of Inhalation Reference
NOAEL	3	Hyperplasia, hypertrophy, or atrophy, but no change in organ weights.	Concentrations and
NOAEL/LOAEL	4	Hyperplasia, hypertrophy, or atrophy, with changes in organ weights.	Inhalation Dosimetry
LOAEL	5	Reversible cellular changes including cloudy swelling, hydropic change, or fatty changes.	Environmental Criteria and Assessment Office/ORD/US EPA.
(LO)AEL ^b	6	Degenerative or necrotic tissue changes with no apparent decrement in organ function.	EPA/600/8-90/066F
(LO)AEL/FEL	7	Reversible slight changes in organ function.	October 1994
FEL	8	Pathological changes with definite organ dysfunction that are unlikely to be fully reversible.	
FEL	9	Pronounced pathologic changes with severe organ dysfunction with long-term sequelae.	
FEL	10	Death or pronounced life shortening.	

*Adapted from DeRosa et al. (1985) and Hartung (1986).

"The parentheses around the "LO" in the acronym "LOAEL" refer to the fact that any study may have a series of doses that evoke toxic effects of rank 5 through 7. All such doses are referred to as adverse effect levels (AELS). The lowest AEL is the (LO)AEL.

A Structured Approach*



This framework proposes: 1) A standard set of definitions of key terms used to describe the overall outcome of toxicity studies and 2) A structured approach to assist in the consistent interpretation of studies (*e.g.* discriminating between adverse and nonadverse health effects)

* Lewis *et al.*, *Toxicologic Pathology* **30(1)**: 66-74 (2002)

FIGURE 2.—Structured approach to evaluating the outcome of toxicology studies.

A Structured Approach*

Figure 1: Structured approach to evaluating the outcome of toxicology studies



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Discriminating Factors*: "A" and "B"

Discriminating factors 'A' are used to differentiate a difference from control values that has arisen by chance from one that is a treatment-related effect. A difference is less likely to be an effect of treatment if:

- There is no obvious dose response;
- it is due to finding(s) in one or more animals which could be considered 'outlier(s)';
- measurement of the endpoint under evaluation is inherently imprecise;
- it is within normal biological variation (i.e. within the range of historical control values or other reference values);
- there is a lack of biological plausibility (i.e. inconsistent with class effects, mode of action, or what is otherwise known or expected of the test substance).

Step 2 - Is the treatment-related effect adverse?

Discriminating factors 'B' are used to differentiate a non-adverse effect of treatment from an adverse effect. An effect is less likely to be adverse if:

- There is no alteration in the general function of the test organism or of the organ/tissue affected;
- it is secondary to other adverse effect(s);
- it is an adaptive response;
- it is transient;
- · severity is limited e.g. below thresholds of concern;
- effect is isolated or independent, i.e. changes in other parameters usually associated with the effect of concern are not observed;
- effect is not a precursor, i.e. the effect is not part of a continuum of changes known to progress with time to an established adverse effect;
- it is a consequence of the experimental model.

* ECETOC Technical Report No. 85 *Recognition of, and Differentiation Between, Adverse and Non-Adverse Effects in Toxicology Studies* (2002).

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A Structured Approach*: Summary

3. INTERPRETATION OF TOXICOLOGICAL DATA: A STRUCTURED APPROACH

It is generally recognised that evaluating the outcome of complex multi-endpoint toxicology studies is not a straightforward exercise. A comprehensive assessment of toxicological data will involve:

- Expert opinion and judgement, where experience is required to integrate complex and diverse information into a coherent interpretation.
- Recognition that effects may represent a continuum, a threshold or an all-or-nothing response.
- Recognition that in hazard characterisation there are often areas open to interpretation, where description of the outcome in terms of weight of evidence and overall level of concern may be more appropriate and informative than simply commenting on whether an effect is considered to be adverse or not.

^{*} ECETOC Technical Report No. 85 *Recognition of, and Differentiation Between, Adverse and Non-Adverse Effects in Toxicology Studies* (2002).

Example Observation: Liver Hypertrophy*

- 1. Is there histological evidence of structural degenerative or necrotic changes such as:
 - hepatocyte necrosis, fibrosis, inflammation, and *steatotic vacuolar degeneration
 - biliary/oval cell proliferation, degeneration, fibrosis, and cholestasis
 - necrosis and degeneration of other resident cells within the liver
- 2. In the absence of histological changes, using a weight-of-evidence approach, is there clinical pathology evidence of hepatocyte damage characterized by a dose dependent and biologically significant and consistent increase in at least *two* liver parameters:
 - at least ×2 to ×3 increase in ALT (EMEA 2010, FDA 2009; HED Guidance Document 2002) or
 - a biologically significant change in other biomarkers of hepatobiliary damage (ALP, AST, γGT, GLDH, etc.)
 - a biologically significant change in another clinical pathology marker indicating liver dysfunction (albumin, bilirubin, bile acids, coagulation factors, cholesterol, triglycerides etc.).

If the above mentioned adverse criteria are *not* observed, then increases in liver organ weight and liver cell hypertrophy due to enzyme induction can be considered as an adaptive response to a xenobiotic and of little relevance to man.

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ATSDR: Non-Adverse Health Effects*

No Adverse Effects

- Weight loss or decrease in body weight gain of less than 10%.
- Changes in organ weight of non-target organ tissues that are not associated with abnormal morphologic or biochemical changes (see guidance on "Assessment of Organ Weight Change").
- Increased mortality over controls that is not significant (p>0.05).
- Some adaptive responses (see guidance on "Assessment of Hepatic Adaptive Responses").

* Agency for Toxic Substances and Disease Registry (ATSDR). 2007. *Guidance for the Preparation of a Twenty First Set Toxicological Profile*. http://www.atsdr.cdc.gov/toxprofiles/guidance/set_21_guidance.pdf

Clinical Chemistry – Lab Animals

TABLE 31.1 PLASMA BIOCHEMICAL VALUES IN COMMON LABORATORY ANIMALS

		-	Guinea		
	Mice	Rats	Pigs	Hamsters	Rabbits
Glucose (mg/dL)	196-278	114-143°	89-95	65-144	89-144
Urea nitrogen (mg/dL)	21-26	16-19°	22-25	14-30	14-23
Creatinine (mg/dL)	0.5	0.5-1.4	1.4	0.5-0.6	0.8-2.9
Uric acid (mg/dL)	-	1.3-2.8	- Sector S	1.3-5.1	1.1-1.2
Total protein (g/dL)	5.0-7.0	6.4-8.5°	4.8-5.6	1.3-5.1	50-85
Albumin (g/dL)	3.0-4.0	4.1-5.4	2.4-2.7	3.2-4.3	30-34
Calcium (mg/dL)	7.9-10.5	10.5-13.0	9.6-10.7	10.4-12.4	130-150
Phosphorus (mg/dL)	5.6-9.2	5.0-13.0	5.0	5.0-8.0	5.6-9.2
Sodium (mEq/L)	138-186	143-150	122-125	128-145	114-156
Potassium (mEq/L)	5.3-6.3	5.3-7.5	4.9-5.1	47-53	44-74
Chloride (mEq/L)	99-108	85-102	92-97		89-120
Cholesterol (mg/dL)	—	36-100	_	94-237	22-69
Total bilirubin (mg/dL)	_	0.0-0.6	0.0-0.9	0.2-0.5	0.0-0.7
Alkaline phosphatase (IU/L)	66-262	70-132°	66-74	8-202	<120
Alanine aminotransferase (IU/L)	40-189	26-37ª	39-45	28-107	<100
Aspartate aminotransferase (IU/L)	77-383	40-53°	46-48	53-202	<100
Lactate dehydrogenase (IU/L)	—	63-573		94-237	<200
Creatine kinase (IU/L)	—	6-309	—	469-1553	<275

Data compiled from the ranges of mean values without consideration of strain, age, gender, and method of blood collection as published in Loeb WF, Quimby FW, eds. The clinical chemistry of laboratory animals. New York: Pergamon Press, 1989;417–509.

^a Values obtained from samples collected via the orbital sinus of Sprague-Dawley rats.

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³⁴ Source: Campbell, TW. Clinical Chemistry of Mammals: Laboratory Animals and Miscellaneous Species. In: *Veterinary Hematology and Clinical Chemistry*, MA Thrall, Editor. Lippincott Williams & Wilkins (2004)

GHS Classification* for STOT-SE & STOT-RE

- Specific Target Organ Toxicity (Single Exposure)
 - Chapter 3.8
- Specific Target Organ Toxicity (Repeated Exposure)
 - Chapter 3.9
- "Reliable evidence associating [single or repeated] exposure to the substance with a consistent and identifiable toxic effect demonstrates support for classification."





Example: Toxic Effects that Provide Support for GHS Classification of STOT-RE

- (a) Morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, or due to the overwhelming of the de-toxification process by repeated exposure;
- (b) Significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, hearing and sense of smell);

- (c) Any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters;
- (d) Significant organ damage that may be noted at necropsy and/or subsequently seen or confirmed at microscopic examination;
- (e) Multifocal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity;
- Morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g. severe fatty change in the liver);
- ³⁶ (g) Evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.

Example: Effects that Do Not Provide Support for GHS Classification of STOT-RE

- (a) Clinical observations or small changes in bodyweight gain, food consumption or water intake that may have some toxicological importance but that do not, by themselves, indicate "significant" toxicity;
- (b) Small changes in clinical biochemistry, haematology or urinalysis parameters and/or transient effects, when such changes or effects are of doubtful or minimal toxicological importance;
- (c) Changes in organ weights with no evidence of organ dysfunction;
- (d) Adaptive responses that are not considered toxicologically relevant;
- (e) Substance-induced species-specific mechanisms of toxicity, i.e. demonstrated with reasonable certainty to be not relevant for human health, should not justify classification.

STOT-RE: Category 1 and 2 Classification

Table 3.9.1: Guidance values to assist in Category 1 classification

Route of exposure	Units	Guidance values (dose/concentration)	🗭 = LOAEL
Oral (rat)	mg/kg bw/d	≤ 10	
Dermal (rat or rabbit)	mg/kg bw/d	≤ 20	
Inhalation (rat) gas	ppmV/6h/d	≤ 50	
Inhalation (rat) vapour	mg/litre/6h/d	≤ 0.2	
Inhalation (rat) dust/mist/fume	mg/litre/6h/d	≤ 0.02	

Table 3.9.2: Guidance values to assist in Category 2 classification

Route of exposure	Units	Guidance value range 📃	🔶 = Loael
		(dose/concentration)	
Oral (rat)	mg/kg bw/d	$10 < C \le 100$	
Dermal (rat or rabbit)	mg/kg bw/d	$20 < C \leq 200$	
Inhalation (rat) gas	ppmV/6h/d	$50 < C \le 250$	
Inhalation (rat) vapour	mg/litre/6h/d	$0.2 < C \le 1.0$	
Inhalation (rat) dust/mist/fume	mg/litre/6h/d	$0.02 < C \le 0.2$	

Guidance is for substances

Guidance assumes data are from 90-day repeat dose studies in experimental animals Classification of mixtures: Use substance guidance (above) or see 3.9.3

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Effects Not Supporting Classification*

"Evidence indicating that R48 should not be applied. The use of this risk phrase is restricted to 'serious damage to health by prolonged exposure'. A number of substance-related effects may be observed in both humans and animals that would not justify the use of R48. These effects are relevant when attempting to determine a no-effect level for a chemical substance. Examples of well documented changes which would not normally justify classification with R48, irrespective of their statistical significance, include:

- a) Clinical observations or changes in bodyweight gain, food consumption or water intake, which may have some toxicological importance but which do not, by themselves, indicate 'serious damage';
- b) small changes in clinical biochemistry, haematology or urinalysis parameters which are of doubtful or minimal toxicological importance;
- c) changes in organ weights with no evidence of organ dysfunction;
- d) adaptive responses (e.g. macrophage migration in the lung, liver hypertrophy and enzyme induction, hyperplastic response to irritants). Local effects in the skin produced by repeated dermal application of a substance which are more appropriately classified with R38 'irritating to skin';
- e) where a species-specific mechanism of toxicity (e.g. specific metabolic pathways) has been demonstrated."

 xicity (e.g. specific metabolic pathways)
 Effects

 in Toxic

 studies

 R48/25; R48/24; R48/23

H372 Causes damage to organs through prolonged or repeated exposure	R48/25; R48/24; R48/23
H373 May cause damage to organs through prolonged or repeated exposure	R48/20; R48/21; R48/22

Non-adverse health effects as per the EC (1993)

*Source: ECETOC Technical Report No. 85 Recognition of, and Differentiation Between, Adverse and Non-Adverse Effects in Toxicology Studies (2002).

Summary

- Repeat-exposure (dose) toxicity studies can be very complex in terms of results:
 - Multiple endpoints/parameters (clinical observations, clinical chemistry, hematological, urinalysis, gross and histopathology...) that could indicate an effect(s) on which to classify under GHS

· Adverse vs. Non-Adverse Health Effects (and Adaptive Effects)

 The appropriate health hazard classification of substances and mixtures is the foundation of an effective, informative and scientifically defensible hazard communication program.

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