European Commission

JRC SCIENCE FOR POLICY REPORT

Identification of Substances of Very High Concern (SVHC) under the 'equivalent level of concern' route (REACH Article 57(f)) – neurotoxicants and immunotoxicants as examples

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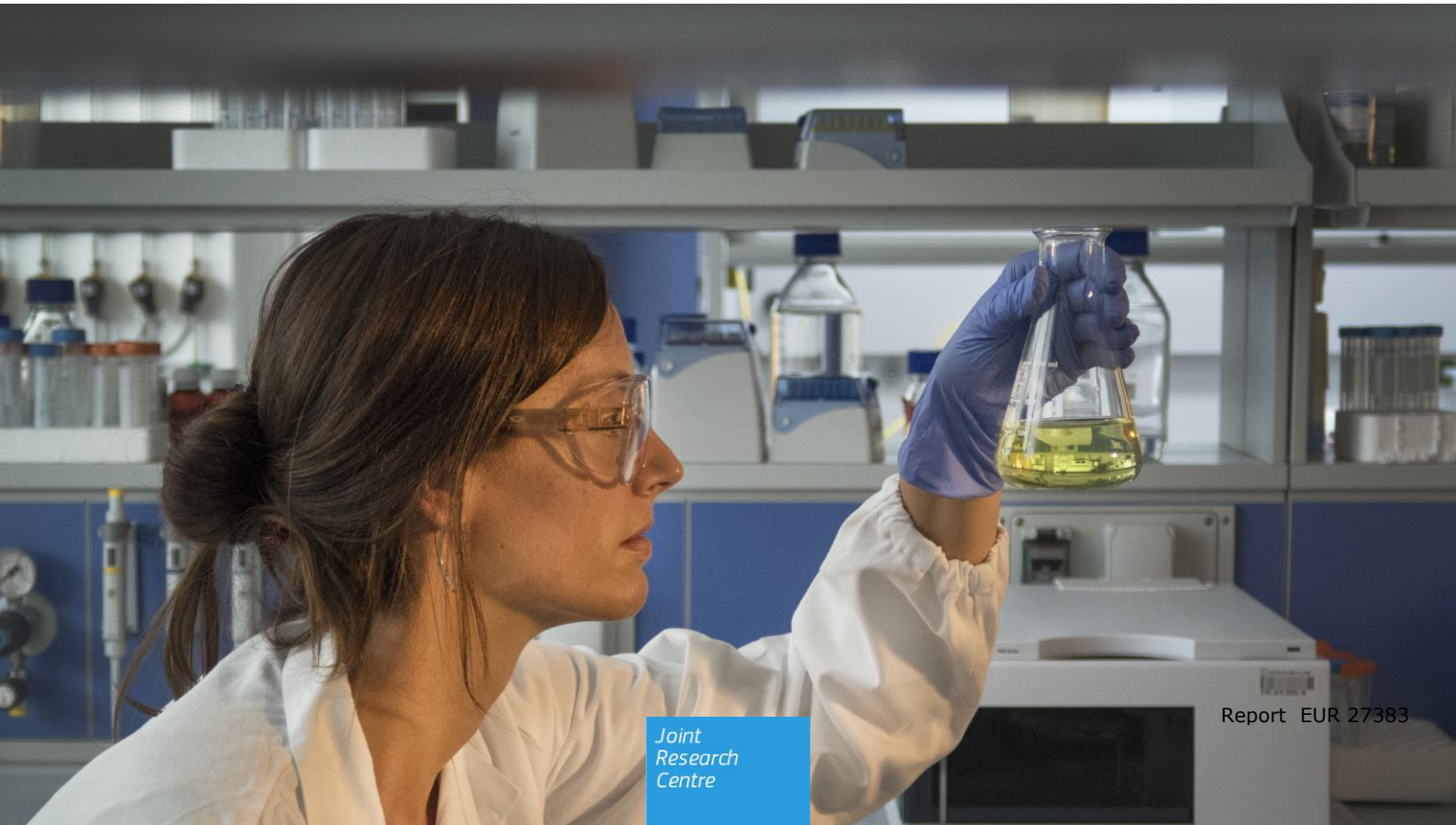
2015



Report EUR 27383

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JRC96572

EUR 27383 EN

ISBN 978-92-79-50319-1 (PDF)

ISSN 1831-9424 (online)

doi:10.2788/080027

Luxembourg: Publications Office of the European Union, 2015

Abstract
This report analyses whether substances classified for specific target organ toxicity (STOT) according Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging, in particular those causing immunotoxic or neurotoxic effects, would be eligible to be identified as substances of very high concern (SVHC) under the 'equivalent level of concern' route set out in Article 57(f) of REACH Regulation (EC) No 1907/2006 (Registration, Evaluation, Authorisation and Restriction of Chemicals). This document attempts to identify, characterise and compare the 'level of concern' that exists for immunotoxic and neurotoxic substances with that of CMRs (carcinogens, mutagens and/or reproductive toxicants). The comparison considers the seriousness, irreversibility and delay of hazardous effects, together with other factors, such as the quality of life affected, consequences for society and the possibility to derive a safe concentration.

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August 2015

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Preface

This report has been prepared in the frame of an Administrative Arrangement between the Directorate-General Environment (DG ENV) and the Joint Research Centre (JRC), Institute for Health and Consumer Protection (IHCP) on 'Scientific and technical support to safety assessment of chemicals'. It analyses whether substances classified for specific target organ toxicity (STOT) according Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging, in particular those causing immunotoxic or neurotoxic effects, would be eligible to be identified as substances of very high concern under the 'equivalent level of concern' route set out in Article 57(f) of REACH Regulation (EC) No 1907/2006 (Registration, Evaluation, Authorisation and Restriction of Chemicals). After an internal review by JRC-IHCP and DG ENV, a draft version of this report was circulated to participants of the 'Sensitiser and other equivalent level of concern substances (ELoC) Coordination Group (SCG)' organised by ECHA. Comments received from the SCG were taken into consideration in this final version.

We would like to thank the SCG experts and the colleagues from DG ENV, ECHA and JRC, especially from the Systems Toxicology and Chemical Assessment and Testing Units, for their useful comments.

Executive summary

Article 57 of REACH Regulation (EC) No 1907/2006 (Registration, Evaluation, Authorisation and Restriction of Chemicals) specifies the criteria for substances to be included in Annex XIV, the 'List of Substances Subject to Authorisation' under REACH, also known as Substances of Very High Concern (SVHC). According to Article 57 (a-e) carcinogens, mutagens, and chemicals toxic for reproduction (CMR) as well as substances which are persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) may be considered SVHC. In addition endocrine disruptors and other chemicals which give rise to an equivalent level of concern (ELoC) (equivalent to CMRs, PBT or vPvB), and for which there is scientific evidence of probable serious effects to human health or environment can be identified as SVHC on a case-by-case basis, as stated in Article 57(f) of this regulation. There is currently no specific guidance or established criteria available and therefore case studies for specific toxicity endpoints are being prepared to facilitate this ELoC comparison.

This paper aims at analysing whether substances classified in the Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging (CLP) as Specific Target Organ Toxicity (STOT) Category 1 and Category 2 (single or repeated exposure (STOT-SE or STOT-RE)), particularly immunotoxicants and neurotoxicants, fulfil the proposed criteria to support the identification of these compounds as SVHC according to Art. 57(f). It attempts to identify, characterise and compare the level of concern that exists for immunotoxicants and neurotoxicants with that of CMRs. This assessment is based on the consideration that in certain cases it may be demonstrated that the impacts caused by immunotoxicants and neurotoxicants on the health of the affected individuals and on the society as a whole, can be comparable to those elicited by CMRs. To determine an equivalent level of concern to CMRs, the comparison assessment focuses on the seriousness, irreversibility and delay of health effects but considers also other factors such as effects on quality of life, societal concerns or the possibility to derive a 'safe concentration'.

The assessment suggests that some substances showing immunotoxic or neurotoxic effects could present an 'equivalent level of concern' to CMRs and potentially be identified as SVHC according to the Art. 57(f) route in REACH. For both, neurotoxic and immunotoxic substances a case-by-case justification considering the hazard assessment and evidence of an equivalent level of concern is necessary. Societal concern and impairment of quality of life can be a consequence of exposure to neurotoxicants or immunotoxicants particularly for a vulnerable part of the population, including children and elderly as well as people with a challenged immune system, which may be more sensitive to lower concentrations. A particular challenge is that for both, immunotoxic and neurotoxic effects, the relationship between chemical exposure and effect may not always be obvious. In general, identification of SVHC that exert immunotoxic effects is more complex than of substances with neurotoxic effects, due to the lack of EU methods or OECD test guidelines.

Neurotoxicity or immunotoxicity effects occurring at dose ranges that are critical for the respective STOT-SE/RE classification (category 1 and 2) should be primarily considered for SVHC identification. This paper underlines the importance of a harmonised classification as a STOT-SE/RE substance for these specific effects before starting any SVHC identification. However for the 'equivalent level of concern' evaluation and justification also other factors need to be taken into account on a case-by-case basis.

1. Background

The European Commission is committed to *have all relevant currently known Substances of Very High Concern (SVHC) included in the Candidate list (Annex XIV o REACH) by 2020*[1]. The objective is to determine the relevance of the substances already recognised as SVHC and to identify potential new ones. To achieve this target, the Commission and ECHA with the support of Member States Competent Authorities for REACH drafted a Roadmap in which screening of dossiers and evaluation of Risk Management Options (RMO) strategies are fundamental steps in the identification of relevant SVHC that later on may be subject to an authorisation process.

According to Article 57 of the REACH Regulation (EC) No 1907/2006, substances of very high concern are substances that meet the criteria for classification as carcinogens, mutagens, and chemicals toxic for reproduction (CMR) as well as substances which are persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) (Art.57 (a-e)). Article 57 (f) applies to endocrine disruptors and other chemicals *which give rise to an equivalent level of concern* to CMRs or PBTs or vPvBs, for *which there is scientific evidence of probable serious effects to human health or environment […] and which are identified on a case-by-case basis.* Such substances may be identified as SVHCs *in accordance with the procedure set out in Article 59*.

This paper aims at analysing whether substances classified for specific target organ oxicity (STOT), in particular those causing immunotoxic or neurotoxic effects, are eligible to be identified as substances of very high concern. This document is thus in line with the objective of the Roadmap 2020 drafted by the EC to *define a process or methodology […] on how to assess the different groups of potential SVHCs*, in particular with the purpose to identify relevant SVHCs falling under Article 57 (f) of REACH.

This document introduces the steps for the general identification of SVHC and the proposed factors to assess an equivalent level of concern to CMRs. Thereafter it presents the criteria for STOT-SE/RE classification and indicates a list of methods and technical challenges found in the hazard identification process for neurotoxicants and immunotoxicants. The main part of the document is a systematic assessment of the proposed ELoC factors to evaluate their applicability in the identification of neurotoxicants and immunotoxicants as SVHC. Annex I includes an extended description of criteria for STOT-SE/RE classification according CLP; Annex II describes adverse health effects caused by immunotoxicants and neurotoxicants. Finally, examples for chemicals with potential neurotoxic effects are presented in Annex III.

[1] Council of the European Union: Roadmap for SVHCs identification and implementation of REACH Risk Management measures from now to 2020 http://register.consilium.europa.eu/pdf/en/13/st05/st05867.en13.pdf

2. General identification and assessment of SVHC

The process of identification of SVHC is, in general, based on intrinsic hazard properties of substances. Thus, when possible, the consultation of the harmonised classification and criteria provided in Annex I of CLP (Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures) is useful as a starting point.

For substances under Art. 57(a-e), hazard identification and characterisation in accordance with CLP regulation is the first step in the process of SVHC identification. In the case of the substances following the route Art. 57(f), it would, however, not be a straightforward task. Along with the hazard identification, a substance may be identified as SVHC if it is demonstrated, on a case-by-case basis, that it shows adverse effects on human health of equivalent level of concern (ELoC) compared to CMR effects[2]. Art. 57 (f) was envisaged as a safety net to control other potential risks than those covered by Art. 57(a-e), and little guidance is provided to determine the level of concern and assist the regulators in the identification of SVHC substances based on equivalent concern.

With the purpose to provide support in this task, a workshop was organised in March 2012 by the German institutions, BfR (Federal Institute for Risk Assessment) and BAuA (Federal Institute for Occupational Safety and Health)[3]. In this meeting, some considerations and principles were discussed in the context of establishing a harmonised and general concept for SVHC identification according to the mentioned Art. 57 (f) route[4]. Factors identified as relevant for the identification of a substance as a SVHC and for comparison of the ELoC with CMR substances were: seriousness of effect (e.g. partly or non-reversible effects, delayed time lag following exposure or postnatal developmental toxicity), strength of evidence, relevance for humans and potency.

The first group of substances discussed for identification as SVHC via Art. 57 (f) route (excluding endocrine disruptors) were chemicals with respiratory and skin sensitising properties. ECHA prepared a discussion paper in which they discussed sensitisers, as a potential group to be identified as SVHC and concluded that the relevant factors mentioned in the BfR/BAuA workshop could be used to demonstrate an 'equivalent level of concern' to CMRs[5]. The document also advocated getting a harmonised classification and labelling under CLP before suggesting identification of the substance concerned as SVHC, and in case a harmonised and general concept cannot be followed, a case-by-case justification for each individual substance to be identified as SVHC was suggested by different partners during the discussions.

[2] In this document ELoC is only considered in relation to human health concerns.

[3] BfR and BAuA workshop on 'REACH Article 57 (f): Non-Endocrine Disrupting Human Health Hazards Leading to SVHC Identification'. (http://www.bfr.bund.de/cm/343/reach-article-57-buchstabe-f-non-endocrine-disrupting-human-health-hazards-leading-to-svhc-identification.pdf)

[4] http://www.baua.de/en/Topics-from-A-to-Z/Hazardous-Substances/Workshops/REACH-2012.html

[5] Identification of substances as SVHCs due to equivalent level of concern to CMRs (Article 57(f)) – sensitisers as an example; http://echa.europa.eu/documents/10162/19126370/dp_sensitisers_final_en.pdf

3. Identification of STOT-SE/RE classified substances as SVHC

The current document focuses on substances classified as specific target organ toxicity (STOT) and the objective is to analyse whether such compounds present hazard characteristics that may give rise to an ELoC to CMRs. This is based on the assumption, that any severe, delayed and/or persistent damage to organs or organ systems falling under the STOT-SE/RE criteria may give rise to ELoC to CMRs. This document focuses specifically on immunotoxic and neurotoxic properties as these effects are considered of specific concern.

3.1. STOT classification

Specific target organ toxicity, according to the CLP Regulation (Annex I: section 3.8.1.1 and 3.9.1.1), is defined *as specific, [non-lethal][6] target organ toxicity arising from a single [or repeated] exposure to a substance or mixture[7]. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed and not specifically addressed by* [other hazard categories].

Specific target organ toxicity can occur by any route that is relevant for humans and encompasses several adverse effects that may be severe, delayed and/or irreversible such as haemotoxicity, hepatotoxicity, nephrotoxicity, neurotoxicity and immunotoxicity, but also transient effects such as respiratory tract irritation and narcotic effects. Effects falling under another hazard category such as acute toxicity, skin corrosion/irritation, serious eye damage/eye irritation, respiratory or skin sensitisation, germ cell mutagenicity, carcinogenicity, reproductive toxicity or aspiration toxicity are covered by other classification criteria and, therefore they should not be classified as STOT.

Substances presenting STOT effects are classified differently depending on whether these adverse effects are detected after single or repeated exposure and consequently would be classified as STOT-single exposure (STOT-SE) or STOT-repeated exposure (STOT-RE), respectively (Table 1). In some cases, toxic effects classified as STOT-SE could be mistaken as acute toxicity effects and care should be taken to avoid 'double classification'. The main criterion to differentiate these two classifications is lethality; classification for acute toxicity is assigned to lethal effects, while non-lethal severe effects in a single organ or biological system but also generalised changes of a less severe nature involving several organs, delayed and/or irreversible toxic effects after single exposure are classified as STOT-SE.

[6] This criterion only applies for STOT-SE (single exposure).

[7] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02008R1272-20131201&from=EN

Table 1 Classification and labelling for specific target organ toxicity after single and repeated exposure

Hazard class	Hazard category	Abbreviation	GHS pictogram	Signal word	Hazard code	Hazard statement
Specific target organ toxicity Single exposure	Category 1	STOT SE 1		Danger	H370	Causes damage to organs (or state all organs affected, if known) (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)
	Category 2	STOT SE 2		Warning	H371	May cause damage to organs (or state all organs affected, if known) (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)
	Category 3	STOT SE 3		Warning	H335	May cause respiratory irritation
					H336	May cause drowsiness or dizziness
Specific target organ toxicity Repeated exposure	Category 1	STOT RE 1		Danger	H372	Causes damage to organs (state all organs affected, if known) through prolonged or repeated exposure (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)
	Category 2	STOT RE 2		Warning	H373	May cause damage to organs (state all organs affected, if known) through prolonged or repeated exposure (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)

As the range of possible STOT effects is broad, this document will be centred on the evaluation of substances with neurotoxic (including effects on special senses and effects on the peripheral nervous system) or immunotoxic effects as potential candidates to be identified as SVHC (Annex II: Adverse health effects caused by immunotoxicants and neurotoxicants). This is motivated by the fact that these effects, besides inducing direct toxic effects, may lead to cognitive and behavioural impairment or increased sensibility sensitivity to infectious or other diseases, which may have considerable consequences on the quality of life and create societal cost in terms of the profound health compromise.

This document focuses on substances classified as STOT-SE/RE 1 and 2, which cause or may cause serious damage to organs through single or repeated exposure. Substances showing reversible and less serious effects such as respiratory irritation and drowsiness or dizziness effects, classified as STOT-SE 3, are not considered for SVHC identification.

3.1.1. Identification of hazard information for STOT-SE/RE substances

The identification of human health hazard generally comes from animal studies or where available from human experience/incidents. Information may also be obtained using read-across from structurally-related substances and from appropriate *in vitro* or *in silico* models, if applicable. Animal tests involve single or repeated exposure via any relevant route of exposure[8]. According to CLP guidance, acute toxicity studies in animals may provide hazard information about toxic effects of a substance on target tissues/organs that reach the classification as STOT-SE. Furthermore, STOT-RE effects can be determined using repeated dose animal studies treated during 28 day, 90 day or lifetime studies (up to 2 years), together with the information provided by other long-term studies (carcinogenicity, neurotoxicity or reproductive toxicity). During the assessment of potential STOT substances, adverse effects in more than one organ should be considered, and due to the difficulties to interpret some results, in most cases expert judgement is required to follow a weight-of-evidence approach considering all the data available.

Standard oral (but not standard dermal or inhalation) 28-day and 90-day toxicity studies include endpoints capable of detecting evidence of neurotoxic and immunotoxic effects. In addition, in 2011 OECD adopted as a Test Guideline the 'Extended One Generation Reproductive Toxicity study (EOGRTS)' (OECD TG 443). This study is designed not only to provide information on reproductive toxicity but also includes other endpoints such as developmental neurotoxicity, developmental immunotoxicity, and endocrine-mediated toxicity. Immunotoxic and neurotoxic effects identified using OECD TG 443, TG 426 (Developmental Neurotoxicity Study) or other developmental studies capable of identifying these effects as described below, would however be considered as developmental effects leading to a hazard classification under the category of reproductive toxicants in CLP regulation and would thus already be considered as SVHC according to Art 57 (c). Therefore, for the identification of neurotoxic and immunotoxic hazard other studies may be necessary to further investigate the same effects[9]. In this regard, international guidance documents are available to assist in the evaluation of available data and to recommend tests to determine STOT specific effects such as neurotoxicity and immunotoxicity[10,11,12,13].

3.1.2. Identification of hazard information for neurotoxicants

Indicators of neurotoxicity include clinical observations, a functional observational battery, motor activity assessment and histopathological examination of the spinal cord and the sciatic nerve.

The identification of chemicals with potential to cause damage on the developing brain is performed following OECD TG 426 (Developmental Neurotoxicity Study, mostly in rats) and on the matured nervous system according to TG 424 (Neurotoxicity Study in rodents). The delayed neurotoxicity

[8] ECHA guidance to Regulation (EC) No 1272/2008 (CLP Regulation) http://echa.europa.eu/documents/10162/13562/clp_labelling_en.pdf
[9] ECHA Guidance on information requirements and Chemical Safety Assessment (http://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf)
[10] Harmonization Project Document No. 10: Guidance for immunotoxicity risk assessment for chemicals (WHO/IPCS)
[11] OECD series on testing and assessment: Number 20, Guidance Document for Neurotoxicity Testing (2004)
[12] EPA Risk Assessment Forum: Guidelines for Neurotoxicity Risk Assessment (1998)
[13] Environmental Health Criteria 223. Neurotoxicity risk assessment for human health: principles and approaches, 2001 (http://www.inchem.org/documents/ehc/ehc/ehc223.htm#_223241000)

study in hens (OECD TG 419) is specifically designed to be used in the assessment and evaluation of the neurotoxic effects of organophosphorus substances.

To obtain information on potential neurotoxic effects of chemicals, the OECD Guidance document for neurotoxicity testing (2004)[14] suggested other OECD Test Guidelines including those for single dose toxicity (e.g. OECD TG 402[15], TG 403[16], TG 420[17], TG 423[18] and TG 425[19]), repeated dose toxicity (e.g. OECD TG 407[20] and TG 408[21]), as well as Test Guidelines specifically developed for the study of neurotoxicity in adult and young laboratory animals [OECD Test Guidelines (TG 418 and TG 419) for Delayed Neurotoxicity of Organophosphorus Substances and OECD Test Guideline for Developmental Neurotoxicity (TG 426), OECD Test Guideline for Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test (TG 422)] [22]. The Biocidal Products Regulation (BPR) (EU) No 528/2012[23] also gives recommendations on the toxicological information required to support the approval of an active substance. In the case of the evaluation of neurotoxicity, the legal text mentions the preferred test species (rat, except for delayed neurotoxicity where adult hens are suggested) or specific tests such as cholinesterase activity test[24].

It is important to indicate that neurotoxicity not only includes effects on the central nervous system but also serious damage (morphological or functional or both) of the eye (retina, eye nerve), ear (hearing), and effects affecting the sense of smell and the peripheral nervous system. Examinations in the standard OECD test guideline are often not sufficient for the hazard characterisation or even identification of these effects.

3.1.3. Identification of hazard information for immunotoxicants

Indicators of immunotoxicity include changes of haematological parameters, serum globulin levels, organ weights alterations and/or histopathological changes in immune organs such as spleen, thymus, lymph nodes and bone marrow.

In the case of immunotoxicants, no specific OECD TG guidelines or EU methods are available to characterise immunotoxic properties and information on immunosuppression, immunostimulation or autoimmune diseases may not always be derived from available standard test guidelines. The information requirements in the BPR (EU) (EU) No 528/2012 regarding immunotoxicity mention direct observation (e.g. clinical cases) to assess *any pathogenicity and infectiveness to humans and*

[14] http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono(2004)25
[15] OECD (1987),Test No. 402: Acute Dermal Toxicity, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.
[16] OECD (2009),Test No. 403: Acute Inhalation Toxicity, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.
[17] OECD (2002),Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.
[18] OECD (2002),Test No. 423: Acute Oral toxicity - Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.
[19] OECD (2008),Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.
[20] OECD (2008),Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.
[21] OECD (1998),Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.
[22] OECD series on testing and assessments (Number 20): Guidance Document for neurotoxicity testing. ENV7JM7MONO(2004)25.
[23] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02012R0528-20140425&from=EN
[24] Biocidal Product Regulation (BPR, Regulation (EU) No 528/2012).

other mammals under conditions of immunosuppression. Information from immunosuppression tests included in other international guidance documents (e.g. EMA (ICH Topic 8 from 2006)[25], EPA (OPPTS 870.7800 from 1998)[26]) may also be considered.

Due to the lack of EU/OECD methods, a better identification and distinction of effects associated with the immune system (e.g. immunosuppression and immunostimulation) may only be derived from enhanced examinations included in repeated dose toxicity studies[27]. Also, as for the neurotoxicants, the outcome of the assessment of developmental immunotoxicity in EOGRTS may be informative on potential effects of immunotoxicants in adults.

For specific immunological effects such as respiratory and skin sensitisation, partly validated tests are available. These have, however, a separate classification, and will therefore not be addressed in this document; the criteria to identify these substances as SVHC have been previously examined in the above-mentioned ECHA discussion paper.

Specific effects and their potency, supporting the classification for Category 1 and 2 of STOT-SE/RE in CLP guidance, together with specific adverse effects of immunotoxicants and neurotoxicants are discussed in more detail in classification in this document.

[25] ICH Topic S 8 Immunotoxicity Studies for Human Pharmaceuticals. CHMP/167235/2004.

[26] Health Effects Test Guidelines: OPPTS 870.7800 Immunotoxicity [EPA 712–C–98–351]: http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0156-0049

[27] Schulte, Agnes et.al. "Two Immunotoxicity Ring Studies According to OECD TG 407—Comparison of Data on Cyclosporin A and Hexachlorobenzene." Regulatory Toxicology and Pharmacology 36**,** 12–21 (2002).

4. 'Level of Concern' comparison

The first step in the identification of a chemical as SVHC under Art. 57(f) is the hazard characterization, i.e. in the current case the identification as an immunotoxicant or neurotoxicant. This should enable a decision on a classification of the substance, if required. Hazard characterisation and classification *per se* however are not sufficient and an 'equivalent level of concern' compared to carcinogenic, mutagenic or reproductive toxic substances needs to be also demonstrated.

The level of concern thus takes into account other factors in addition to hazardous properties, as stated in the ECHA discussion paper on the *Identification of substances as SVHCs due to equivalent level of concern to CMRs (Article 57(f)) – sensitisers as an example* are: *[…] the seriousness of the effects, the often irreversible nature of the effects, the consequences for society and the difficulty in performing concentration-based risk assessments (except for reproductive effects)*[28]*.* Furthermore, the OECD Guidance document for neurotoxicity testing (2004) suggests that *the level of concern of the neurotoxic effects should be based on type, severity, number and either full or partial reversibility of the effects. Chemicals which produce a clear and consistent pattern of neurotoxicity at lower dose levels than other organ/system toxicity are generally of higher concern than chemicals which produce only a few unrelated effects.*

To compare the level of concern of immune- and neurotoxicants with CMRs, this document builds on ECHA's discussion paper, in which the factors considered suitable to identify as to whether a substance with respiratory or skin sensitising properties may be identified as a SVHC have been evaluated. The same factors are discussed here to evaluate the level of concern for adverse effects elicited by exposure to immunotoxicants and neurotoxicants. The evaluation of these factors for CMRs presented in ECHA's paper (see text in *italics*) is included also in this document for comparative purposes. Furthermore, for clarity, the same outline as in ECHA's discussion paper has been followed:

- Health effects (Section 4.1)
  - Type of possible health effects
  - Irreversibility of health effects
  - Delay of health effects
- Other factors (Section 4.2)
  - Quality of life affected
  - Societal concern
  - Is derivation of a 'safe concentration' possible?

Table 2 summarises the comparison based on these relevant factors among CMRs, immunotoxicants and neurotoxicants. This table also includes results from the previous analysis of ELoC on respiratory sensitisers, presented in ECHA's document.

[28] Identification of substances as SVHCs due to equivalent level of concern to CMRs (Article 57(f)) – sensitisers as an example (http://echa.europa.eu/documents/10162/13657/svhc_art_57f_sensitisers_en.pdf)

Table 2. 'Level of Concern' comparison between CMRs, Respiratory Sensitisation, Immunotoxicity and Neurotoxicity[29]

	C & M	R	Respiratory Sensitisation	Immunotoxicity	Neurotoxicity
Possible serious health effects?	**YES –** ▪ Serious & permanent organ dysfunction ▪ Inheritable defects ▪ Could lead to death	**YES –** ▪ Serious & permanent organ dysfunction ▪ Malformations or death in (unborn) children	**YES –** ▪ Serious & permanent organ dysfunction ▪ Permanent impairment of lung functions ▪ Could lead to death	**YES –** ▪ Potential for serious & permanent organ dysfunction ▪ Morphological abnormalities indicative for impairment/functional changes of immune system; increase of neoplastic diseases, and infections. Induction and increase of autoimmune diseases and allergic processes ▪ Could lead to death	**YES –** ▪ Potential for serious & permanent organ dysfunction ▪ Permanent impairment/functional changes of central or peripheral nervous system, other organs/organ systems including CNS depression and effects on special senses (motor, sight, hearing, sense of smell) as well as learning and memory.
Irreversibility of health effects[30]***?***	**YES –** ▪ Irreversible effects	**YES –** ▪ Irreversible effects	**YES –** ▪ Induction phase of sensitisation ▪ Elicitation phase of sensitisation -can lead to irreversible lung dysfunction	**YES –** ▪ Effects can be irreversible	**YES –** ▪ Effects can be irreversible, depending on dose and time of exposure (especially during susceptible life stages: postnatal development and aging) and/or half-life in body
Delay of health effects?	**YES –** ▪ Long delay until effects manifest	**YES –** ▪ Medium delay until effects manifest	**YES –** ▪ Long/medium delay between induction and elicitation phases	**YES –** ▪ Delay (medium to long) until effects manifest possible	**YES –** ▪ Delay (medium to long) until effects manifest possible (depending on half-life/accumulation in body)

[29] This table is a modification from Table 1 in ECHA's paper on sensitisers (see the reference in footnote 28).

[30] Based on discussion if only irreversible effects classify as serious it was concluded that also some CR effects could be reversible (BfR and BAuA workshop on 'REACH Article 57 (f): Non-Endocrine Disrupting Human Health Hazards Leading to SVHC Identification').

	C & M	R	Respiratory Sensitisation	Immunotoxicity	Neurotoxicity
Quality of life impaired?	**YES** – ▪ Long-term illness limiting possibility of living a normal working and private life ▪ Possible mental/ psychological impacts	**YES** – ▪ Children with developmental effects may need life-long medication/ support in their daily life ▪ Life of parents also affected (emotional investment, care, financial costs)	**YES** – ▪ Long-term illness limiting the possibility of living a normal working and private life ▪ Require long-term medication ▪ Re-training of affected staff	**YES** – ▪ Long-term illness limiting possibility of living a normal working and private life ▪ Possible mental/ psychological impacts (due to related effects such as reduced defense, cancer or autoimmune diseases)	**YES-** ▪ Long-term illness and reduced productivity limiting possibility of living a normal working and private life ▪ Possible mental/ psychological impacts (due to effects on peripheral or central NS and special senses)
Societal concern?	**YES** – ▪ Widespread concern about cancer ▪ Cost implications for society in terms of healthcare	**YES** – ▪ Widespread concern about adverse effects in children ▪ Cost implications for society in terms of healthcare ▪ Disability	**YES** – ▪ Cost implications for society in terms of healthcare and retraining ▪ Associated with disability	**YES** – ▪ Widespread concern about increase of infections or other related diseases ▪ Cost implications for society in terms of healthcare (increase morbidity/premature mortality) ▪ Disability	**YES –** ▪ Widespread concern about effects on the adult nervous system ▪ Cost implications for society in terms of healthcare (increased risk of antisocial behavior as well as morbidity/premature mortality) ▪ Disability
Is derivation of a 'safe concentration' possible?	**NORMALLY NO -** ▪ 'Zero risk' only possible where no exposure	**NORMALLY YES -** ▪ Possible to determine a safe concentration	**NO –** ▪ Difficult to establish the threshold dose for induction and elicitation ▪ Derivation of safe concentration is not routinely possible	**Potentially YES –** ▪ Possible to determine a safe concentration for certain substances ▪ Difficult to establish a safe concentration for certain end-points (U-shaped dose-response relationships) for certain substances	**Potentially YES -** ▪ Possible to determine a safe concentration for certain substances for adult neurotoxicity ▪ Difficult to establish a safe concentration for certain end-points (U-shaped dose-response relationships) for certain substances

4.1. Health Effects

4.1.1. Type of possible health effects

The initial point to identify a substance as SVHC is to evaluate whether the adverse effects exerted by the exposure to that substance are serious and relevant to humans. Classification under CLP regulation may provide information on the hazardous properties of substances and guidance to evaluate whether these effects may have a serious impact on human health and could be considered as equivalent level of concern to CMR substances (Cat. 1A/B).

- In the case of carcinogens and mutagens, exposure to these substances has the potential to cause serious adverse health effects in a proportion of the population i.e. serious and permanent organ *dysfunction, inheritable defects and/or death.*

- *In the case of reproductive toxicants (development), exposure has the potential to cause serious adverse health effects in a proportion of the population i.e. serious and permanent organ dysfunction, defects and/or death.*

- In the case of **immunotoxicants**, exposure has the potential to cause serious adverse health effects in (a proportion of) the population. In principle, immunotoxicants can cause immunosuppression, immunostimulation or an abnormal immune response (such as autoimmunisation) (see Annex II: Adverse health effects caused by immunotoxicants and neurotoxicants). Immunosuppression effects can be linked to an increase of susceptibility to infectious diseases as well as to a decrease of the response to immunisation, potentially leading to an increased number of infections cases in the general population. In this regard, increases in the incidence of colds or influenza in occupational settings, as in the case of lead were reported[31]. Furthermore, exposure to certain immunotoxicants has been related to an increased incidence of neoplastic diseases or the development of certain autoimmune diseases; i.e. several studies have associated the occupational exposure to crystalline silica to rheumatoid arthritis, scleroderma, or systemic lupus erythematosus [32,33]. Exposure to some organic solvents has been also described as a risk factor for developing autoimmune diseases such as scleroderma-like syndrome[34,35]. However, the findings from different studies on chemical-induced autoimmunity are not always consistent and further research may be needed to make definitive conclusions on these effects, at this point of time. Also it is extremely difficult to link the development of autoimmune diseases to a specific chemical, due to the complex nature of autoimmune diseases, i.e. genetic disposition and

[31] Horiguchi, S., et al. "Frequency of cold infections in workers at a lead refinery." Osaka city medical journal 38.1 (1992): 79.
[32] Environmental health criteria 236. Principles and methods for assessing autoimmunity associated with exposure to chemicals (WHO, 2006).
[33] Cooper, Glinda S., Frederick W. Miller, and Dori R. Germolec. "Occupational exposures and autoimmune diseases." International immunopharmacology 2.2 (2002): 303-313.
[34] Cooper, Glinda S., et al. "Evidence of autoimmune-related effects of trichloroethylene exposure from studies in mice and humans." Environ Health Perspect 117.5 (2009): 696-702.
[35] Barragán-Martínez, Carolina, et al. "Organic solvents as risk factor for autoimmune diseases: a systematic review and meta-analysis." PloS one 7.12 (2012): e51506.

environmental factors. Besides, there may be long lag time before the disease is manifested, hence the link to the potential causal agent may be impossible to make. Cases of chemical exposure and immunostimulation effects have been also reported in the literature. For example, repeated dose 28-day oral toxicity studies in rats treated with hexachlorobenzene showed histomorphologic alterations, an increased lymphocyte migration activity, indicative of immunostimulation[36].

- In the case of **neurotoxicants**, exposure of humans may lead to changes in sensory function, motor function, and changes in the performance of learned behaviour, memory and cognitive impairment. The exposure to some neurotoxicants has also been discussed to be related to the development of progressive neurodegenerative diseases, e.g. Alzheimer's and Parkinson-like diseases; however, further research has been suggested to confirm this association since data on this topic is very scarce. In fact, some of the observed neurological disorders may actually originate from exposure during development. Although the development of the nervous system continues postnatally, developmental neurotoxicity for the purposes of hazard classification and the CLP regulation would fall in the category of reproductive toxicants and thus already be considered as SVHC according to Art 57 (c).

4.1.2. Irreversibility of health effects

In the ECHA discussion paper on sensitisers, the irreversibility of effects is listed as a supportive criterion to identify a substance as SVHC, based on the fact that changes in the structure and/or function of the organ could be permanent. Irreversibility is estimated by the absence of a full recovery or by an incomplete recovery within the foreseen recovery periods of the respective test guidelines.

- *In the case of carcinogens and mutagens, adverse health effects e.g. development of cancer may lead to death or irreversible ill health.*

- *In the case of reproductive toxicants (development), adverse health effect may be present in the form of irreversible malformations in children.*

- In the case of some **immunotoxicants**, reversibility of adverse effects has been observed and once the exposure to the substances ceases, the immune system is able to return to its prior status[37]. However, one of the concerns of immunosuppression effects is its association with an increased risk of cancer due to the failure of the immune system or to a higher risk of developing virus-induced neoplasias[38]. Even though there is evidence of a link between certain chemicals and immunosuppressive effects, it is important to note that some authors have reported that the data to support a causal relationship between the association of

[36] Schulte, Agnes, et al. "Two immunotoxicity ring studies according to OECD TG 407—comparison of data on cyclosporin A and hexachlorobenzene." Regulatory Toxicology and Pharmacology 36.1 (2002): 12-21.
[37] Guidance for immunotoxicity risk assessment for chemicals (IPCS harmonization project document; no. 10).
[38] Luster, Michael I., et al. "Relationships between chemical-induced immunotoxicity and carcinogenesis." Drug information journal 30.1 (1996): 281-286.

certain cancers and chemicals with immunotoxic potential is insufficient, especially following exposure of adults[39]. Occupational exposure to chemicals such as certain solvents, crystalline silica or certain pesticides may influence the development of systemic autoimmune disease leading to irreversible effects[40]. These associations have been reported only in epidemiological or animal studies and are not always strong and clear.

- In the case of **neurotoxicants**, adverse effects may be reversible or irreversible. The nervous system is known for having a functional reserve capacity. Its ability to return to the state prior to exposure, as well as its ability to compensate and adapt for neurotoxic insults is however limited and depends on the targeted cells, subcellular structures or neurotransmitters and the internal dose leading to neurotoxicity. Its capacity of regeneration following neuronal cell degeneration/death is absent or very limited and even though effects after single exposure to certain neurotoxicants may disappear when the exposure ceases, as in the case of certain organic solvents, this fact may not discard the presence of injuries or cell death. Also, in some cases exposure to neurotoxicants has been associated with chronic and irreversible diseases, e.g. long-term occupational exposure to certain organic solvent may induce encephalopathy, an irreversible damage to the central nervous system (mild to severe cognitive impairment that affects memory, attention, and psychomotor functions). Also chronic exposure to neurotoxicants may be linked with the development of neurodegenerative diseases[41], which entail the irreversible loss of neuronal structure and functions, also defined as neurodegeneration[42]. For example, certain neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease have been discussed to be associated with exposure to metals or pesticides. Occupational exposure to heavy metals, e.g. manganese, iron, copper, mercury, zinc, and lead has been associated with neurological symptoms with similarities to Parkinson's disease such as weakness, bent posture, whispering speech, limb tremor, and salivation[43,44]. Furthermore, drinking water and occupational exposure to aluminium[45,46] and other metals such as mercury and lead have been suggested to be a risk factor in Alzheimer's disease or the development of neuropathological (neurofibrillary tangles and senile plaques) and clinical symptoms (presenile dementia, reduced mental test scores) similar to those of Alzheimer's disease[47].

[39] Veraldi, Angela, et al. "Immunotoxic effects of chemicals: A matrix for occupational and environmental epidemiological studies." American journal of industrial medicine 49.12 (2006): 1046-1055.

[40] Cooper, Glinda S., Frederick W. Miller, and Dori R. Germolec. "Occupational exposures and autoimmune diseases." International immunopharmacology 2.2 (2002): 303-313.

[41] HPA CHaPD 001: Review of Environmental Chemicals and Neurotoxicity: Focus on Neurological Diseases (HPA, UK, 2007)

[42] Cannon, Jason R., and J. Timothy Greenamyre. "The role of environmental exposures in neurodegeneration and neurodegenerative diseases." Toxicological Sciences 124.2 (2011): 225-250.

[43] Gorell, J. M., et al. "Occupational exposures to metals as risk factors for Parkinson's disease." Neurology 48.3 (1997): 650-658.

[44] Olanow, C. W. "Manganese-induced parkinsonism and Parkinson's disease." Annals of the New York Academy of Sciences 1012.1 (2004): 209-223.

[45] Flaten, Trond Peder. "Aluminium as a risk factor in Alzheimer's disease, with emphasis on drinking water." Brain research bulletin 55.2 (2001): 187-196.

[46] Graves, Amy B., et al. "Occupational exposures to solvents and aluminium and estimated risk of Alzheimer's disease." Occupational and environmental medicine 55.9 (1998): 627-633.

[47] Liu, Guijian, et al. "Metal exposure and Alzheimer's pathogenesis." Journal of structural biology 155.1 (2006): 45-51.

Aluminium has been also described as a potent neurotoxicant and the causal agent of encephalopathy in patients that receive dialysis due to chronic renal failure[48,49].

During the BfR/BAuA workshop, some stakeholders commented that although some adverse effects may be reversible over time, the potential damage and social impact as a result of these temporary effects cannot be excluded (e.g. prolonged periods of illness, lower performance or accidents at work).

In certain exposure scenarios it could be relevant to take also into account the impact that reversible effects may have on human health and on the determination of the level of concern. Reversible effects such as headache, dizziness, decrease of concentration, movement disorders in occupational settings in which operating dangerous machinery is required may raise a higher level of concern for their fatal consequences. These effects are normally a response to exposure concentrations eliciting acute effects. However, in some cases the reversibility of such effects may be so slow that these effects may raise a level of concern similar to irreversible effects. In general, a complicating issue is that the permanence (irreversibility) of effects (especially for immunotoxicity) may often not be demonstrated in standard guideline studies, and possibly be derived only from epidemiological data and generic medicinal knowledge on these aethiopathologies.

4.1.3. Delay of health effects

Toxic effects of some substances may be only detected after a long period of time between the exposure and manifestation of the adverse effects. In this case, risk management measures may be not taken in time, and individuals might be exposed for a long period to chemicals for which potential health effects and risks are unknown. Delay of effects, although not a specific relevant factor for the identification of SVHCs, could be an important aspect to establish higher levels of concern.

In addition, delay of effects of chemicals could be an important feature during early postnatal stages of life, in which systems affected are immature and effects can be expressed or observed only after a long period of time. In this case, it is often difficult to relate this effect to a chemical exposure and therefore it won't be recognise to be of concern. Toxicity testing may help in the identification of delayed effects, and taking appropriate risk management measures on time could avoid the potential risk posed by these chemicals.

- *In the case of carcinogens and mutagens, there are usually long delays before adverse effects manifest themselves.*

[48] Alfrey, Allen C., Gary R. LeGendre, and William D. Kaehny. "The dialysis encephalopathy syndrome: possible aluminum intoxication." New England Journal of Medicine 294.4 (1976): 184-188.
[49] Wills, MichaelR, and John Savory. "Aluminium poisoning: dialysis encephalopathy, osteomalacia, and anaemia." The Lancet 322.8340 (1983): 29-34.

- *In the case of reproductive toxicants (development) there can be some delay before adverse effects manifest themselves.*

- In the case of **immunotoxicants**, depending on the mode of action and the time of exposure, both immediate and long delayed adverse effects could be expected. Depending on the dose and the observed effects, the immune system may have a capacity to return to normal status after exposure to immunotoxicants; however, immune dysfunction due to early postnatal exposure or during immune system maturation, may increase the later-life risk to certain autoimmune diseases and other health effects[50] or immune dysfunction (e.g. by decreased defense against infections) may become obvious much later than the primary immunosuppressive effect. As mentioned previously, an increased risk of cancer might also be a potential delayed effect of exposure to immunosuppressant chemicals or a co-morbid effect of substances with chemical-induced autoimmunity effects[51].

- In the case of **neurotoxicants**, exposure of children to certain chemicals with neurotoxic effects may lead to delayed and permanent effects such as learning disabilities, sensory and memory deficits, and cognitive developmental delays. Also long-term low-level exposure has been related to a decrease in performance in neuropsychological functions; an increase of persistent developmental disorders, delays in cognitive development and attention deficit/ hyperactivity disorder[52]. In adults, short-term exposure to some organic solvents has been linked to transient symptoms that generally disappear when the exposure ceases; however, long-term exposure may result in the delayed effects (observed for organic solvents or when the neurotoxicants accumulates in the nervous tissues). A well-known example of delayed effects of neurotoxicants are the symptoms after exposure to some organophosphates which is also called 'organophosphate induced delayed polyneuropathy', a rare neurodegenerative disease that is characterised by the central and peripheral nervous system degeneration, followed by symptoms as ataxia and paralysis that may appear after 3 weeks after exposure. Other toxic effects associated with exposure to organophosphates are the cholinergic syndrome (sweating, lacrimation, salivation, respiratory difficulties, tremors, ataxia), and the intermediate syndrome (respiratory difficulties due to malfunctions of respiratory muscles) that appear not delayed after exposure. Depression symptoms have been also reported after long-term occupational exposure to certain chemicals, such as certain pesticides [53] or carbon disulphide[54,55].

[50] These effects would be considered as developmental effects and therefore be considered as SVHC.

[51] Dietert, Rodney R. "Role of developmental immunotoxicity and immune dysfunction in chronic disease and cancer." Reproductive Toxicology 31.3 (2011): 319-326.

[52] Grandjean, Philippe, and Philip J. Landrigan. "Developmental neurotoxicity of industrial chemicals." The Lancet 368.9553 (2006): 2167-2178.

[53] Bazylewicz-Walczak, B., W. Majczakowa, and M. Szymczak. "Behavioral effects of occupational exposure to organophosphorous pesticides in female greenhouse planting workers." Neurotoxicology 20.5 (1999): 819-826.

[54] Vigliani, Enrico C. "Carbon disulphide poisoning in viscose rayon factories." British journal of industrial medicine 11.4 (1954): 235.

[55] Aaserud, Olaf, et al. "Carbon disulfide exposure and neurotoxic sequelae among viscose rayon workers." American journal of industrial medicine 18.1 (1990): 25-37.

4.2. Other considerations

According to the ECHA discussion paper on sensitisers, other factors of concern to consider the identification of a substance under Art. 57 (f) are the impairment of quality of life, consequences for society and the difficulty to establish dose-response relationships for the risk assessment of these substances.

The social impact of exposure to a substance may be determined at individual level (how the exposure and effects of this substance impacts on the regular life of an affected individual) or at societal level, regarding which are the social and economic implications for the population.

In addition, the possibility to derive safe levels of exposure could be informative to decide the 'level of concern' of a substance and may be taken into consideration on a case-by-case basis for prioritisation. Exposure to chemicals for which safe levels can be derived may be controlled by risk management measures and lead to a lower level of concern.

4.2.1. Quality of life affected

- *In the case of carcinogens and mutagens, possible side-effects such as organ dysfunction can result in the person having to live with a long-term illness, limiting the possibility of living a normal working and private life. Regardless of the prognosis, the negative health effects caused by exposure to carcinogens and mutagens are generally considered to be a 'serious' consequence, as it always has the potential to be fatal.*

- *In the case of developmental toxicants, depending on the effect manifested, the long-term consequences for the infants/person may be very severe and impair the quality of life. Children having developmental effects may need life-long medication and/or support during their daily life. There is also an indirect effect on the quality of life of such children's parents in terms of emotional investment, care and financial resources needed.*

- In the case of **immunotoxicants**, long-term adverse health effects may appear after exposure, i.e. an increase risk to severe infections and/or cancer or irreversible autoimmune diseases. In these cases, the possibilities for affected individuals to live a normal life are limited and quality of life could be impaired for long periods.

- In the case of **neurotoxicants**, exposure may lead to chronic diseases that need life-long treatment[56,57]. Also, at the workplace, exposure to neurotoxicants has been associated with a slow-down of the performance on reaction-time tasks or changes in vision[58,59]. For

[56] Alavanja, Michael CR, Jane A. Hoppin, and Freya Kamel. "Health Effects of Chronic Pesticide Exposure: Cancer and Neurotoxicity* 3." Annu. Rev. Public Health 25 (2004): 155-197.
[57] Kamel, Freya, and Jane A. Hoppin. "Association of pesticide exposure with neurologic dysfunction and disease." Environmental Health Perspectives 112.9 (2004): 950.
[58] Viaene, M. K., et al. "Neurobehavioural effects of occupational exposure to cadmium: a cross sectional epidemiological study." Occupational and environmental medicine 57.1 (2000): 19-27.

example, long-term exposure to white spirit (longer than 10 years) may lead to the impairment of brain function and can therefore be associated with a high risk for the development of a chronic toxic encephalopathy[60].

It is important to note, that certain immunotoxic or neurotoxic effects if detected time after exposure, e.g. during childhood and adolescence, may originate from exposure during *in utero* development and should therefore be classified as neurodevelopmental toxicants (i.e. toxic for reproduction, R). Some substances may be immunotoxic and/or neurotoxic in adults and progeny, possibly at different dose levels. It has been suggested that immunotoxicity and neurotoxicity could occur at lower doses in postnatal developing organisms than in adults (see also 5.2). These effects are not examined in the test guidance used to reach the developmental toxicity classification. Each effect needs proper consideration with regard to the population group and hazard class.

4.2.2. Societal concern

Societal concern is not considered a determining factor for the identification of substances as SVHC but it could provide relevant information about the level of concern of a substance, in particular about its effects at social and economic levels in society.

- *In the case of carcinogens and mutagens, there is widespread concern in society, due to the high prevalence of cancer in the worldwide population and the uncertainty surrounding future effects that may be persistent, e.g. development of cancer and potential death. There can be a high cost of treating affected individuals in society.*

- *In the case of developmental toxicants, the potential adverse health effects on children e.g. severe malformations or restrained intellectual capabilities are of high concern for the society. There can also be a high cost of treating affected individuals in society.*

- In the case of **immunotoxicants,** substances with immunosuppressive mechanisms, which may result in an increased risk of incidence of neoplasms (due to immunosuppression), may give rise to a similar level of concern as carcinogens. Also a decrease in host resistance and an improper response to vaccination are primary effects of concern because of public health implications: the spread and increase of infectious diseases such as cold and influenza generate costs related to increased sick leave or absences from work, costs in health care goods and services, and loss of productivity at the workplace. Furthermore, autoimmune chemical-related diseases are in general chronic; thereby it may imply a lifetime cost for the health system[61].

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[59] Costa, Thiago Leiros, et al. "Long-Term Occupational Exposure to Organic Solvents Affects Color Vision, Contrast Sensitivity and Visual Fields." PloS one 7.8 (2012): e42961.

[60] Nielsen, Gunnar Damgård, Søren Peter Lund, and Ole Ladefoged. "Neurological Effects of White Spirit: Contribution of Animal Studies during a 30-Year Period*." Basic & clinical pharmacology & toxicology 98.2 (2006): 115-123.

[61] Blanciforti, Laura A., and Michael I. Luster. "Considerations in estimating social and economic impacts of immunotoxic agents." Human and Ecological Risk Assessment 12.5 (2006): 888-903.

- In the case of **neurotoxicants**, the levels of concern to chemical exposure are based on the magnitude of effect, duration of the exposure and reversibility of some neurotoxic effects. Of particular concern is the fact that few chemicals have been evaluated for neurotoxicity as well as the lack of research on the relationship between adverse effects and exposure to low concentrations of neurotoxicants and its potential impact on the society[62]. As mentioned, neurotoxic effects at the workplace may be very serious and may lead to significant financial losses resulting from health problems[63].

4.2.3. Is derivation of a 'safe concentration' possible?

The possibility to derive safe concentrations is not a decisive factor during the process to identify a chemical as SVHC, as the identification of SVHC is based on hazard; however, this information could be relevant and may be taken into consideration on a case-by-case basis. Exposure to chemicals whose safety levels can be derived may be controlled by later risk management measures.

- *In the case of non-threshold carcinogens and mutagens, it is only possible to conclude 'zero risk' if there is no exposure. In certain cases, even very small doses of such substances can cause adverse effects, which may only manifest after several years of exposure. Consequently, derivation of a safe concentration is normally not possible, except in few cases of non-genotoxic carcinogens.*

- *In the case of developmental toxicants, it is normally possible to determine a toxicological threshold and consequently a safe concentration.*

- In the case of **immunotoxicants**, it is normally possible to derive a dose-response relationship for immunosuppression; however, the calculation of this dose-relationship depends on the mode of action of the chemical and the considered endpoint. In this regard, and bearing in mind the lack of scientific consensus in this topic, some researches have suggested that certain immunotoxic end-points may follow a non-monotonic dose-response curve, in which effects produced at low doses are opposite to those exerted by high doses (also called 'hormesis effect'). An example for this is the effect of methylmercury in the response of peripheral blood lymphocytes to phytohaemagglutinin, where in rats high-doses produce suppression and low-doses produce stimulation of this parameter[64]. Therefore, 'safe concentrations' may vary not only depending on the substance but also on the immune parameter considered which might be important information in particular cases.

- In the case of **neurotoxicants**, it is possible to establish safe levels of exposure in most of the cases, but the same issues with potential hormesis effects as described above can be found

[62] Bellinger, David C. "Effect modification in epidemiologic studies of low-level neurotoxicant exposures and health outcomes." Neurotoxicology and teratology 22.1 (2000): 133-140.
[63] Callender, T. J., et al. "Social and economic impact of neurotoxicity in hazardous waste workers in Lenoir, North Carolina." Environmental research 73.1 (1997): 166-174.
[64] Omara, Felix Olima, et al. "Immunotoxicity of environmentally relevant mixtures of polychlorinated aromatic hydrocarbons with methyl mercury on rat lymphocytes in vitro." *Environmental toxicology and chemistry* 16.3 (1997): 576-581.

in the literature for some chemicals showing neurotoxic effects including polychlorinated biphenyls or heavy metals such as mercury or aluminium [65,66]. Although a safe concentration may exist theoretically, some neurotoxicants (such as lead)[67] are hazardous at extremely low concentrations that are already exceeded by environmental exposure.

[65] Kuriyama, Sergio Noboru, and Ibrahim Chahoud. "In utero exposure to low-dose 2, 3', 4, 4', 5-pentachlorobiphenyl (PCB 118) impairs male fertility and alters neurobehavior in rat offspring." Toxicology 202.3 (2004): 185-197.

[66] Calabrese, Edward J., and Linda A. Baldwin. "Inorganics and hormesis." CRC Critical Reviews in Toxicology 33.3-4 (2003): 215-304.

[67] http://www.efsa.europa.eu/de/efsajournal/doc/1570.pdf

5. Specific considerations

5.1. Potency

In the previous ECHA discussion paper, potency was discussed as a potential supporting factor, specific for sensitisers, when evaluating the level of concern during the process of identification of SVHC. Potency may be a factor to be considered since potent immunotoxicants or neurotoxicants may evoke adverse effects at lower exposure levels. Similarly to sensitisers, potent immunotoxicants or neurotoxicants are suggested to be prioritised over less potent toxicants, since less potent toxicant will give rise to a lower concern than toxicants with high potency. Toxicity of substances, and as consequence potency, is based on a description of the dose-effect relationship values which could be used as a measurement of toxic potential and to set exposure limits[68]. However, in contrast with the case of sensitisers (e.g. EC3 derivation from murine local lymph node assay test (LLNA) values) [69], there are no established values or tests for potency evaluation of immune and neurotoxicants. The guidance values as defined in the CLP criteria[70] for STOT-SE/RE category 1and 2 (see classification could be used for hazard identification and classification but also as potency indication.

5.2. Groups at risk to immunotoxicant and neurotoxicant exposure

In order to evaluate the social impact of some chemicals, it is important to take into consideration the group of the population at higher health risk due to exposure to these substances. In certain cases, some individuals of the population such as children and pregnant women are usually considered groups at risk[71]. In other cases, determinant characteristics of a group of people could lead to a higher risk for particular chemicals. Age, an immunocompromised state or genetic susceptibility are some of these characteristics.

5.2.1. Age

Age-related difference to exposure to chemicals could be linked not only to differences in exposure and accumulation over time but also differences in susceptibility to substances. In the elderly population, immunosenescence, an age-related dysfunction of the immune system, together with other factors such as malnutrition and age-associated physiological changes have been related to an increase of the susceptibility to infectious diseases, and a decrease in the response to vaccination[72,73,74]. Also, it has been suggested that the elderly could be more susceptible to chemical

[68] Environmental Health Criteria 223. Neurotoxicity risk assessment for human health: principles and approaches (WHO, 2001).
[69] EC3 value is the concentration of a chemical required to elicit a threefold stimulation of proliferation in draining lymph nodes in the murine local lymph node assay. This value is intrapolated from the dose response curve.
[70] Guidance on the Application of the CLP Criteria; http://echa.europa.eu/documents/10162/13562/clp_en.pdf
[71] Effects in these groups may be classified as reprotoxicants.
[72] Wick, Georg, and Beatrix Grubeck-Loebenstein. "The aging immune system: primary and secondary alterations of immune reactivity in the elderly." Experimental gerontology 32.4 (1997): 401-413.
[73] Harmonization Project Document No. 10: Guidance for immunotoxicity risk assessment for chemicals (WHO/IPCS).
[74] Gavazzi, Gaëtan, and Karl-Heinz Krause. "Ageing and infection." The Lancet infectious diseases 2.11 (2002): 659-666.

insults and have a reduced capacity to respond to new toxic perturbations, and to compensate previous exposures. Previous exposure to neurotoxicants has been related with the development of some neurodegenerative diseases in the aged population.

5.2.2. Developmental immune and neurotoxicity

The focus of this paper is immunotoxicity and neurotoxicity in adults; however, in order to understand more the consequences of these chemicals it is necessary to take into consideration some information on developmental immune- and neurotoxicity, even though these effects are specifically covered under toxicity for reproduction. Effects may be diagnosed a long period after birth, and therefore it is an important factor to be discussed in this paper.

It is not explicit if developmental neurotoxicity and immunotoxicity occurring solely as a result of exposure during childhood (and not prenatal or via lactation) is covered by developmental toxicity. CLP Regulation 1272/2008 (Annex I, 3.7.1.4) states that *Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure*. Therefore, the hazard statement of the label covers only the unborn child.

Similar to the elderly, the children's immune and neural system could be more susceptible to chemicals than that of adults. Substances exerting these effects may be referred to as developmental immunotoxicants or neurotoxicants are considered substances toxic for reproduction even though effects may be diagnosed long periods after birth, and therefore it is an important factor to discuss them in this section. Toxicity during development may be more severe due to several factors such as the rapid organ development or/and increased absorption rates; exposure to higher doses (considering dose per kg body weight), and the possibly reduced capacity of detoxification for xenobiotics. In some cases, even low exposure levels may result in long-lasting effects that will become evident with age.

In the case of immunotoxicants, it is generally believed that the immature immune system is more susceptible than the fully mature system and sequels could be more persistent. Maturation of the immune system is a critical and sensitive process that may be affected by exposure to immunotoxicants. Effects during maturation may lead to a lifelong dysfunctional immune system. In children, diseases as leukaemia, influenza, asthma, type 1 diabetes and allergic diseases have been associated with exposure to immunotoxicants during the developmental stage. In contrast, in adults

disturbance of the immune system normally requires higher doses and in comparison to developmental immunotoxicity such effects are more likely to be reversible[75].

In the case of neurotoxicants, the developing brain is more vulnerable than an adult brain. Indeed, exposure during early foetal development can cause brain injury at doses much lower than those affecting adult brain functions. Exposure to chemicals in the environment during early foetal development[76] has been associated with development of neurodevelopmental disorders such as motor and sensory deficits, developmental delays, learning disabilities, attention deficit disorder, mental retardation or autism[77].

5.2.3. Immunodeficiency

Exposure to chemicals that in a healthy population would pose mild or moderate changes in the immune system response may cause severe effects in individuals with a challenged immune system.

5.2.4. Genetic susceptibility and gender

Genetic predisposition in the population may be a factor to consider especially when evaluating chemicals with immunotoxic or neurotoxic effects. Susceptibility to some effects or disorders may increase with the presence of congenital defects, and in some cases the genetic predisposition of an individual could be a determinant factor, as in the case of some chemically-induced autoimmune disorders.

Another influential factor to consider is the gender bias that some immune diseases exhibit. Exposure to immunotoxicants during postnatal early-life stages may produce differences in response depending on the gender. This fact is notable in the case of predominance of some autoimmune diseases[78], particularly in women; in fact it has been reported that under certain conditions (thyroiditis, scleroderma, and systemic lupus erythematous) more than 85% of patients are female[79]. The evaluation of the impact of certain chemicals on the immune system (with exception of sensitisers) is a challenging task that has led to the controversy whether small changes in the immune function may be significant and used to assess the risk of exposure to immunotoxicants in human populations. In the case of neurotoxicants, the sensitivity to neurotoxicants varies across the general population although the reasons for this variability are still unknown[80].

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[75] Luebke, Robert W., et al. "The comparative immunotoxicity of five selected compounds following developmental or adult exposure." Journal of Toxicology and Environmental Health, Part B 9.1 (2006): 1-26.

[76] Effects associated with exposure during early foetal stage are covered by reproductive toxicity.

[77] Grandjean, Philippe, and Philip J. Landrigan. "Developmental neurotoxicity of industrial chemicals." The Lancet 368.9553 (2006): 2167-2178.

[78] Environmental health criteria 236. Principles and methods for assessing autoimmunity associated with exposure to chemicals (WHO, 2006).

[79] Cooper, Glinda S., Frederick W. Miller, and Dori R. Germolec. "Occupational exposures and autoimmune diseases." International immunopharmacology 2.2 (2002): 303-313.

[80] Vahter, Marie, et al. "Gender differences in the disposition and toxicity of metals." Environmental research 104.1 (2007): 85-95.

6. Conclusions

An evaluation of relevant factors to compare an 'equivalent level of concern' to carcinogenic, mutagenic or reprotoxic substances has been performed for substances classified as STOT-SE/RE (category 1 and 2), particularly for substances showing immunotoxic or neurotoxic effects. This evaluation follows the criteria proposed by different stakeholders as considered previously in the ECHA's discussion paper on sensitisers, and includes the following factors: the type of possible health effects, the irreversibility and the delay of such effects.

The evaluation suggests that some substances showing severe immunotoxic or neurotoxic effects following single or repeated exposure could present an 'equivalent level of concern' as CMRs and potentially be identified as SVHC according to the Art. 57(f) route in REACH. For both, neurotoxic and immunotoxic substances a case-by-case justification considering hazard assessment and evidence of an equivalent level of concern is necessary.

Neurotoxic effects include motor and sensor activity alteration, effects on reflex action or effects on cognition. After acute exposure, these effects may be transient, and therefore, some substances causing these effects may not be considered as potential SVHC candidates according to Article 57(f); however, long-term exposure with slowly reversible effects or delayed effects may lead to irreversible damage of the nervous system resulting in serious chronic diseases, e.g. encephalopathy, neurodegenerative diseases, and cognitive impairment.

Also some chemicals causing immunotoxic effects could be seen as potential SVHC candidates. Immunosuppression is normally reversible but long-term exposure may result in secondary effects, e. g., increased risk of infections or development of tumours. Also, a number of autoimmune diseases have been associated with long-term chemical exposure or suggested to arise as delayed effects following an exposure during a critical time window; however, the identification of these chemicals may be problematic due to the very complex nature of these diseases.

In both, immunotoxic and neurotoxic effects, the relationship between chemical exposure and effect may not always be obvious. In general, identification of SVHC that exert immunotoxic effects is more complex than of substances with neurotoxic effects. The reason is, that no specific EU methods or OECD test guidelines are available to characterise immunotoxic properties and information on immunosuppression, immunostimulation or autoimmune diseases is mainly retrieved from non-standard tests.

As stated in Art. 57 (f), the only determining factor for the identification of substances as SVHC, is *the existence of scientific evidence of probable serious effects, which give rise to an equivalent level of concern to CMR substance*. However, other factors as discussed in the BfR/ BAuA workshop on REACH Article 57(f) and as presented in the ECHA discussion paper on sensitisers may provide relevant information about the level of concern of a substance to be taken into consideration in the case-by-case evaluation of these chemicals, such as the impairment of quality of life, consequences for society and the difficulty in performing risk assessments.

In this regard, societal concern and impairment of quality of life can be a consequence of exposure to neurotoxicants or immunotoxicants particularly for vulnerable population. In addition, in some cases the derivation of safe threshold levels could be difficult for both neurotoxicants and immunotoxicants; however in general it is possible and risks can be controlled under different risk management options. Potency, age, genetic susceptibility and gender are considerations that may be taken into account when evaluating the level of concern. In this case, groups at higher risk to immunotoxicant and neurotoxicant exposure include children and elderly as well as people with a challenged immune system, which may be more sensitive to lower concentrations.

In conclusion, neurotoxicity or immunotoxicity effects occurring at dose ranges, which are critical for the respective STOT classification (STOT-SE or STOT-RE, category 1 and 2) should be primarily considered for SVHC identification. In agreement with the previous discussion paper on sensitisers, this document underlines the importance of a harmonised classification as STOT substance for these specific effects before starting any SVHC identification, followed by a case-by-case 'equivalent level of concern' evaluation and justification.

Abbreviations

BaUA	Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (German Federal Institute for Occupational Safety and Health)
BfR	Bundesinstitut für Risikobewertung (German Federal Institute for Risk Assessment)
BPR	Regulation (EU) No 528/2012 on Biocidal Products Regulation
CLP	Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of Substances and Mixtures
CMR	Carcinogenic, Mutagenic or Toxic for Reproduction
ECHA	European Chemicals Agency
ELoC	Equivalent Level of Concern
EMA	European Medicines Agency
EOGRTS	Extended One-Generation Reproductive Toxicity Study
EPA	United States Environmental Protection Agency
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
OECD	Organisation for Economic Co-operation and Development
OECD TG	Organisation for Economic Co-operation and Development Test Guidelines
OPPTS	US-EPA Office of Prevention, Pesticides and Toxic Substances

Annex I: STOT classification in CLP and Criteria for STOT classification

STOT classification encompasses several adverse effects that may be severe, delayed and/or irreversible, including haemotoxicity, hepatotoxicity, nephrotoxicity, neurotoxicity and immunotoxicity, but also transient effects as respiratory tract irritation and narcotic effects. As a starting point, JRC assessed the endpoints immunotoxicity and neurotoxicity.

In the Regulation (EC) No 1272/2008 and the *Guidance to the Regulation on classification, labelling and packaging (CLP) of substances and mixtures*, STOT classification for Category 1 and 2 depends on whether the adverse outcomes are significant or relevant, or whether they are severe, thereby having a significant impact on human health (see summary in table below). This evidence could be based on human studies; e.g. case reports, epidemiological studies, medical surveillance and reporting schemes and information from national poisons centres, and/or based on animal studies that on many occasions could be more informative than human cases, since animal studies are conducted under controlled conditions respecting exposure and other experimental settings.

As mentioned in the guidance, weight-of-evidence and expert judgement are required to properly classify a substance under STOT categorisations. CLP regulation provides dose/concentration guidance values to be used as part of a weight-of-evidence evaluation and to assist in the classification of STOT substances in Category 1 or 2 (see Table A.1 below). In addition, STOT-SE and STOT-RE categories are derived from significant data on non-lethal toxic effects from acute toxicity testing and 28 or 90-day repeated-dose studies, respectively[81].

Other relevant evidences from studies in humans and/or animals to be considered as listed in the CLP Regulation 1272/2008 (Annex I: 3.9.2.7.3) to support STOT classification are:

(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, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites.

(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 noted at necropsy and/or subsequently seen or confirmed at microscopic examination.

[81] CLP Guidance value ranges for single-dose exposures (Annex 1: 3.8.2.1.9.3) and for repeated-dose exposure (Annex 1: 3.9.2.9.6., Annex 3.9.2.9.7). ECHA Guidance on the Application of the CLP Criteria. ECHA (2012).

(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity.

(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver).

(g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.

In the next section, specific adverse effects of immunotoxicants and neurotoxicants significant for STOT classification are discussed.

Table A 1. Criteria and guidance values[82] to assist in Category 1 and 2 classifications for STOT-SE and STOT-RE[83]

	STOT-SE Cat. 1	STOT-SE Cat. 2	STOT-RE Cat. 1	STOT-RE Cat. 2
Criteria	Significant toxicity in humans or potential to produce significant toxicity in humans following single exposure	Presumed to have the potential to be harmful to human health following single exposure	Significant toxicity in humans or potential to produce significant toxicity in humans following repeated exposure	Presumed to have the potential to be harmful to human health following repeated exposure
Basis of evidence	Human cases or epidemiological studies or observations in experimental animals with significant and/or severe toxic effect, of relevance to human health	Studies in experimental animals, of relevance to human health	Human cases or epidemiological studies or observations in experimental animals with significant and/or severe toxic effect, of relevance to human health	Studies in experimental animals of relevance to human health
Observations	Significant non-lethal effects observed in acute toxicity testing	Significant non-lethal effects observed in acute toxicity testing	Significant toxic effects observed in 90-day repeated-dose animal studies, in rats (oral) or rabbits (dermal) Generally low exposure concentrations	Significant toxic effects observed in 90-day repeated-dose animal studies, in rats (oral) or rabbits (dermal) Generally moderate exposure concentrations
Proposed guidance values	C ≤ 300 (oral) C ≤ 1000 (dermal) (mg/kg bw)	2000 < C ≤ 300 (oral) 2000 < C ≤ 1000 (dermal) (mg/kg bw)	C ≤ 10 (oral) C ≤ 20 (dermal) (mg/kg bw/d)	10 < C ≤ 100 (oral) 20 < C ≤ 200 (dermal) (mg/kg bw/d)
Proposed guidance values, inhalation (gas)	C ≤ 2500 (gas) (ppmV/4h)	20000 < C ≤ 2500 (gas) (ppmV/4h)	C ≤ 50 (gas) (ppmV/6h/day)	50 < C ≤ 250 (gas) (ppmV/6h/day)
Proposed guidance values, inhalation (vapour) (dust/mist/fume)	C ≤ 10 (vapour) C ≤ 1.0 (dust/mist/fume) (mg/l/4h)	20 < C ≤ 10 (vapour) 5.0 < C ≤ 1.0 (dust/mist/fume) (mg/l/4h)	C ≤ 0.2 (vapour) C ≤ 0.02 (dust/mist/fume) (mg/l/6h/day)	0.2 < C ≤ 1 (vapour) 0.02 < C ≤ 0.2 (dust/mist/fume) (mg/l/6h/day)

[82] Guidance values and ranges for guidance purposes, as part of the weight-of-evidence approach to assist with decisions about classification.

[83] Adapted from: http://echa.europa.eu/documents/10162/13562/clp_en.pdf; and CLP Regulation

Annex II: Adverse health effects caused by immunotoxicants and neurotoxicants

A.2.1. Adverse immune system effects

The immune system is a complex system that provides the body with a proper balance of the immune response, including host defence against pathogens or neoplasias; response to foreign compounds, and recognition and discrimination of self from non-self body components.

Immunotoxicology investigates how substances can interact, alter, and compromise the immune system function contributing to a wide range of adverse effects from mild to severe. Substances can affect the immune system evoking a combination of adverse outcomes including immunosuppression, allergies, hypersensitivity reactions, higher risk to develop autoimmune diseases and dysfunctional anti-inflammatory responses[84]. The seriousness of these effects depends on the mode of action, severity of damage on immune system (including all immune tissues) and vulnerability of the population exposed as well as the time of exposure to the chemicals.

Among the adverse immunotoxic effects, immunosuppression, immunostimulation and autoimmune response have been taken into consideration as potential outcomes to be relevant for identification of SVHC. Immunological effects such as allergies or hypersensitivity reactions are classified under another CLP category, and have been previously discussed.

- Immunosuppression*:*

  Exposure to chemicals with immunosuppressant effects may lead to an increased risk of incidence of neoplastic diseases, an increase of the susceptibility and potential severity of infectious diseases, and/or a decrease in the response and effect of immunisation, having as a possible consequence also the increase in the number of incidence of infection cases in the general population.

  Although an increased risk to certain types of cancer has been linked to a decreased effectiveness of the immune system in eliminating neoplastic cells or virus- related neoplastic events, cancer has also been described as a co-morbid adverse effect related with other chronic immune-based diseases, especially those as a consequence of developmental immunotoxicity. Regarding changes in host resistance (to tumour cells, bacteria, viruses), adverse effects may be mild or severe, even lethal, depending on the exposure characteristics and vulnerability of the population exposed. Usually, in healthy populations, small changes in resistance to infections will lead to mild to moderate adverse effects, whereas they could be severe in individuals with immunodeficiency diseases or under immunosuppressive therapy.

  Cellular changes such as atrophy or decreases in population of T-cell or B-cell compartments can directly be seen in animal studies (detection can be improved by specific methods that

[84] Harmonization Project Document No. 10: Guidance for immunotoxicity risk assessment for chemicals (WHO/IPCS)

are not standard). The proof of dysfunction of the innate, nonspecific immune responses against infections, is rarely available in regulatory datasets. Testing on specific immune responses is even more rarely found.

- Immunostimulation*:*

  Effects due to alterations by immunostimulation are not very well understood, and usually difficult to evaluate; they may exacerbate allergic reactions, worsening autoimmune disease and induce or amplify inflammatory response causing organ damage.

- Autoimmunity*:*

  Autoimmunity and autoimmune diseases result from altered immune responses against self-molecules. The immunological effectors and mechanisms involved in autoimmune reaction are the same of those associated with responses to foreign antigens, including activation of the innate and adaptive immune systems, production of inflammatory mediators and activation of T lymphocytes or the generation of antibodies with specificity for self-antigens.

  A number of chemicals have been identified as potential triggers of autoimmunity and linked to an elevated risk to some systemic autoimmune diseases (scleroderma-like disease, Raynaud phenomenon, and systemic lupus erythematosus). However, the identification of chemical-associated autoimmune effects is challenging since autoimmune diseases are complex disorders, not yet well understood. Studies in animals exposed to substances showing autoimmunogenic potential (e.g. some metals, dioxins, or some pesticides), presented cellular, biochemical and clinical alterations similar to known autoimmune diseases[85].

Evaluation and identification of immunotoxic effects are not easy tasks. Adverse effects of some chemicals have shown clinical features similar to several autoimmune diseases; however, chemicals inducing autoimmunity are difficult to identify due to the lack of validated models and strategies. Furthermore, the immune system is present in nearly each tissue of any organ and alterations may lead to other organ dysfunctions that by default are not considered as cause of a failure of the immune system. Thus, in most cases the application of a weight-of-evidence approach by experts, on the available data is required.

Currently, the identification of immunotoxicity effects is carried out using *in vivo*, repeated-dose toxicity in rats or mice, and *in vitro* studies, from which a broad range of parameters are measured. This list of factors include haematology changes, lymphoid organ weights, histopathology of lymphoid tissues and tissues where immune complexes may deposit, bone marrow cellularity, distribution of lymphocyte subsets and NK-cell activity or primary antibody response to T-cell

[85] Environmental Health Criteria 236. Principles and methods for assessing autoimmunity associated with exposure to chemicals. WHO (2006)

dependent antigen, as well as alterations in the immune function or characterisation of host resistance response [86].

A.2.2. Adverse effects of neurotoxicants

The nervous system is a complex organ system that controls several functions including sensory, motor and cognitive function.

The OECD guidance for "Neurotoxicity Study in Rodents" (TG 424)[87] describes neurotoxicity as *an adverse change in the structure or function of the nervous system, central and peripheral that results from exposure to a chemical, biological or physical agent.* In this definition, an adverse change should be considered any detrimental effect that affects the normal function of the nervous system resulting from single or repeated exposure. Furthermore, a group of experts on neurotoxicity risk assessment for human health from the WHO Environmental Health Criteria Programme have divided neurotoxic effects in two types, structural and functional[88]: *Structural neurotoxic effects are defined as neuroanatomical changes occurring at any level of nervous system organization.* These changes may be indicated by changes in brain morphology or weight, or histologic changes at tissue and cellular level. *Functional changes are defined as neurochemical, neurophysiological or behavioural effects.* These effects include adverse changes in somatic/autonomic, sensory, motor and cognitive function, and may be traced by changes in neurophysiological endpoints such as changes in nerve conduction, sensory potential or electrocephalographic pattern; in behavioural and neurological endpoints such as headache, dizziness, unconsciousness, seizures, cognitive problems, memory problems, and memory loss, behavioural, alteration of motor control, changes in learning, memory or attention.

In a report, published by EFSA in 2012, on Identification of Cumulative Assessment Groups of Pesticides, the authors grouped the effects on the nervous system in effects concerning functional changes related to the motor division (including movement of muscles, effects of locomotion and neuropathy), effects on reflex action, hypertrophy/ hyperplasia in the brain, cell degenerations and neoplasms in the brain[89].

Neurotoxicity not only includes effects on the central nervous system but also neurotoxic effects (morphological or functional or both) on the eye (retina, eye nerve), ear (hearing), the sense of smell and the peripheral nervous system, considered serious damage of organs. A major problem is that examinations in the standard OECD test guideline are often not sufficient for the hazard characterisation or even identification of these effects. Moreover, indications coming from human observations remain only as indicators since a causal link between exposure and effects is often difficult to establish.

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[86] Dietert, Rodney R., ed. Immunotoxicity testing: methods and protocols. Humana Press, 2010.

[87] OECD (1997), Test No. 424: Neurotoxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.

[88] http://www.inchem.org/documents/ehc/ehc/ehc223.htm

[89] External scientific report submitted to EFSA Identification of Cumulative Assessment Groups of Pesticides (National Food Institute, Technical University of Denmark-EFSA): http://www.efsa.europa.eu/en/supporting/doc/269e.pdf

Some effects of short-term or acute exposure may be detected early after the exposure, whereas long-term exposure to relatively low-concentrations may result in impairment of the functional capacity of the nervous system where can only be detected after a long period of after exposure. In the latter case it is not always easy to establish a causal link between exposure and effects, or between patterns of exposure or dose. In these cases, integration of hazard characterisation, dose-response analysis and exposure assessment as well as expert judgment of the data available is necessary.

Annex III: Examples for chemicals with potential neurotoxic effects

A number of chemicals such as some metals, pesticides and organic solvents have been associated with effects on the nervous system. The severity of these effects can range from mild to severe and it is also important to note that the absence or limited capacity of regeneration of the nervous system, especially in some neuronal regions, increases the level of concern of potential changes that can occur in the system. Some of the effects associated with these chemicals are presented in this Annex:

A.3.1. Metals

Long-term exposure to certain metals can lead to neurotoxic effects. Welders have been associated with a higher risk to suffer some neurological dysfunctions than welders not exposed to these metals. For example, some occupational studies in workers in foundries[90] or exposed to welding fumes[91,92] suggested a link between the chronic inhalation to aluminium with pre-clinical mild cognitive disorder which might prelude Alzheimer's disease and evoke delays in reaction time of exposed workers. Also, some epidemiological studies of drinking water with high content of aluminium have suggested the exposure to high levels of this metal as an important risk factor in the pathogenesis of Alzheimer's type disease[93]. Biochemical changes related with exposure to high levels of aluminium such as formation and accumulation of amyloid beta protein, and aggregation of microtubullar protein tau forming neurofibrillary tangles may contribute to the loss of neurons in the hippocampus (symptoms of mild impairment in verbal memory). Aluminium pot-room workers have presented incoordination, intention tremor and cognitive deficits. Other symptoms often reported were loss of balance, memory loss, joint paint, dizziness, numbness and severe weakness. Other study showed the prevalence of neurological symptoms as incoordination and depression.

Exposure to lead has also been associated with decline in cognitive function (after acute or longer-term exposure), contribution to cognitive and behavioural impairments[94] and to induction of neuropathy[95].

The nervous system is the principal target tissue affected by methylmercury in adults. Chronic exposure to organic forms of mercury such as the lipid-soluble form of mercury has been reported to perturb neuromotor, behavioural and cognitive functions[96].

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[90] Polizzi, Salvatore, et al. "Neurotoxic effects of aluminium among foundry workers and Alzheimer's disease." Neurotoxicology 23.6 (2002): 761-774.

[91] Buchta, M., et al. "Longitudinal study examining the neurotoxicity of occupational exposure to aluminium-containing welding fumes." International archives of occupational and environmental health 76.7 (2003): 539-548.

[92] Buchtaa, Mark, et al. "Neurotoxicity of exposures to aluminium welding fumes in the truck trailer construction industry." Environmental Toxicology and Pharmacology 19.3 (2005): 677-685.

[93] Flaten, Trond Peder. "Aluminium as a risk factor in Alzheimer's disease, with emphasis on drinking water." Brain research bulletin 55.2 (2001): 187-196.

[94] Florea, Ana-Maria, and Dietrich Büsselberg. "Occurrence, use and potential toxic effects of metals and metal compounds." Biometals 19.4 (2006): 419-427.

[95] Chuang, Hung-Yi, et al. "The influence of milk intake on the lead toxicity to the sensory nervous system in lead workers." Neurotoxicology 25.6 (2004): 941-949

A.3.2. Pesticides

Exposure to certain pesticides has been associated with an increased risk for behavioural and neurological long-term outcome. For example some insecticides, including certain organophosphates, carbamates, pyrethroids, or organochlorines interfere with chemical neurotransmission or ion channels, and usually cause reversible neurotoxic effects, that could nevertheless be lethal. Exposure to some of these chemicals has been associated with an increased risk to develop Parkinson's disease[97,98]. Parkinson's disease is a progressive degeneration of dopaminergic neurons of the *substantia nigra pars compacta* that project to the striatum and play a role in controlling motor functions. Effects similar to those evoked in Parkinson's disease as rigidity, resting tremors, bradykinesia and postural instability as well as others such as cognitive impairment, sleep disturbances, olfactory dysfunction and depression have been reported after poisoning or episodes of accidentally high exposure to these pesticides[99].

It is important to note that the association between the exposure to pesticides and neurodegenerative diseases as Alzheimer's disease and Parkinson's disease is under scientific debate since epidemiological studies present some evidence of these links but the data available are insufficient to support a causal relationship between the exposure to a particular pesticide or mixture and these diseases.

A.3.3. Organic solvents

Several organic solvents have been identified as having neurotoxic effects. Iregren and Gamberale (1995) investigated experimentally the potential neurotoxicity of different single and mixtures of organic solvents (such as methyl butyl ketone, styrene, toluene, white spirit, or xylene) and also reviewed effects of solvents investigated by other labs (e.g. formaldehyde, acetone or methyl ethyl ketone). The authors concluded that exposure to organic solvents were generally slight, transient, narcotic effects that resulted in prolonged delayed responses in behavioural tests. In the CLP Regulation, these effects are covered by STOT-SE Cat. 3 as transient target organ effects (Annex I: Table 3.8.1) that, *may range from slight dizziness to deep unconsciousness* and it gives as an example the organic solvent toluene to illustrate this category (CLP 3.8.6.1.4 Example 4: Toluene). Furthermore, these authors also noted that the effects resulting from long-term exposure to these chemical in occupational settings were not very specific and mainly diffuse symptoms as fatigue, memory impairment or reduction in intellectual functions[100].

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[96] Jonnalagadda, S. B., and P. V. V. Rao. "Toxicity, bioavailability and metal speciation." Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 106.3 (1993): 585-595.

[97] Wang, Anthony, et al. "The association between ambient exposure to organophosphates and Parkinson's disease risk." Occupational and environmental medicine (2014): oemed-2013.

[98] Costa, Lucio G., et al. "Neurotoxicity of pesticides: a brief review." Frontiers in bioscience: a journal and virtual library 13 (2007): 1240-1249.

[99] Brown, T. P., et al. "Pesticides and Parkinson's disease--is there a link?" Environmental Health Perspectives 114.2 (2006): 156-164.

[100] Iregren, Anders, and Francesco Gamberale. "Human behavioral toxicology: central nervous effects of low-dose exposure to neurotoxic substances in the work environment." Scandinavian journal of work, environment & health (1990): 17-25.

For white spirit (solvent and thinner in paints) the RAC (Risk Assessment Committee)[101], in agreement with the assessments of the International Programme on Chemical Safety (IPCS 1996)[102] and Scientific Committee on Occupational Exposure Limits (SCOEL 2007)[103] concluded in 2011 that long-term exposure (> 10 years) may lead to the impairment of brain function and can therefore be associated with a high risk for the development of a chronic toxic encephalopathy. In their view the corresponding decline in the number of diagnosed CTE (chronic toxic encephalopathy) cases with the decreasing use of solvent-based paints supports the theory of white spirit as the causative agent. RAC decided for a classification with STOT-RE 1 - H372 (Causes damage to the central nervous system through prolonged or repeated exposure). In that case the available animal data were inconclusive and failed to provide a molecular mechanism that could explain the long-term effects. The adverse effects as measured in humans are related to behavioural changes and may be difficult to detect in animals.

Other examples of neurotoxicity include the toxicity to senses. Ethylbenzene[104] was classified for its ototoxicity with H373 (May cause damage to hearing organs through prolonged or repeated exposure). Evidence is provided by one oral animal study, where high doses caused strong hair cell death in cochleae. There are no specific data on neurotoxicity in humans with mono-exposure to ethylbenzene. However, for other aromatic solvents (e.g. for toluene, xylenes or styrene) there is evidence for neurotoxicity in humans and strong experimental evidence for ototoxicity. A high frequency of hearing loss in workers involved in the production of paints and varnishes (containing the following mixture constituents: ethylbenzene, xylene and trimethylbenzene isomers) was described by Sulkowski et al. (2002)[105].

As pointed out in the case of immunotoxicants, the role of experts for the interpretation and weight-of-evidence based determination is essential, as many neurological tests are qualitative and descriptive, which may complicate their evaluation and the comparison of results. According to the US-EPA, during the evaluation of neurotoxicity of pesticides, the overall toxic profile including hazard identification and characterization as well as information on the mode of action may be considered in the weight-of-evidence approach. To establish the toxicological profile for neurotoxicants, various endpoints may be measured including neuropathological, neurochemical, neuropsychological and behavioural characteristics to provide information about potential effects of chemicals on peripheral and central nervous system; these tests evaluate the toxicity in sensorial organs, neuromuscular effects, learning and memory capacity and histopathology changes of the nervous system.

[101] RAC Opinion proposing harmonised classification and labelling at Community level of white spirit (February 2011) (http://echa.europa.eu/documents/10162/13641/adopted_rac_opinion_on_white_spirit_en.pdf)

[102] IPCS (1996). White Spirit (Stoddard Solvent). Environmental Health Criteria 187. International Programme on Chemical Safety, World Health Organization, Geneva. http://www.inchem.org/documents/ehc/ehc/ehc187.htm

[103] SCOEL (2007). Recommendation of the Scientific Committee on Occupational Exposure; Limits for "White Spirit". SCOEL/SUM/87, August 2007.

[104] RAC Annex 1 Background document to the Opinion proposing harmonised classification and labelling at EU level of Ethylbenzene; http://echa.europa.eu/documents/10162/13579/bd_ethylbenzene_final_pri_clean_en.pdf

[105] Sulkowski, W.J., Kowalska, S., Matyja, W., Guzek,W., Wesolowski, W., Szymczak, W., Kostrzewski, P. (2002): Effects of occupational exposure to a mixture of solvents to the inner ear: a field study. Int. J. Occup. Med. Environm. Health 15, 247-256

LB-NA-27383-EN-N

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ISBN 978-92-79-50319-1

Publications Office