第40回日本毒性学会学術年会
「シンポジウム11」
免疫毒性の最近の潮流 (S11-5)

WHO化学物質の免疫毒性リスク評価ガイダンスについて

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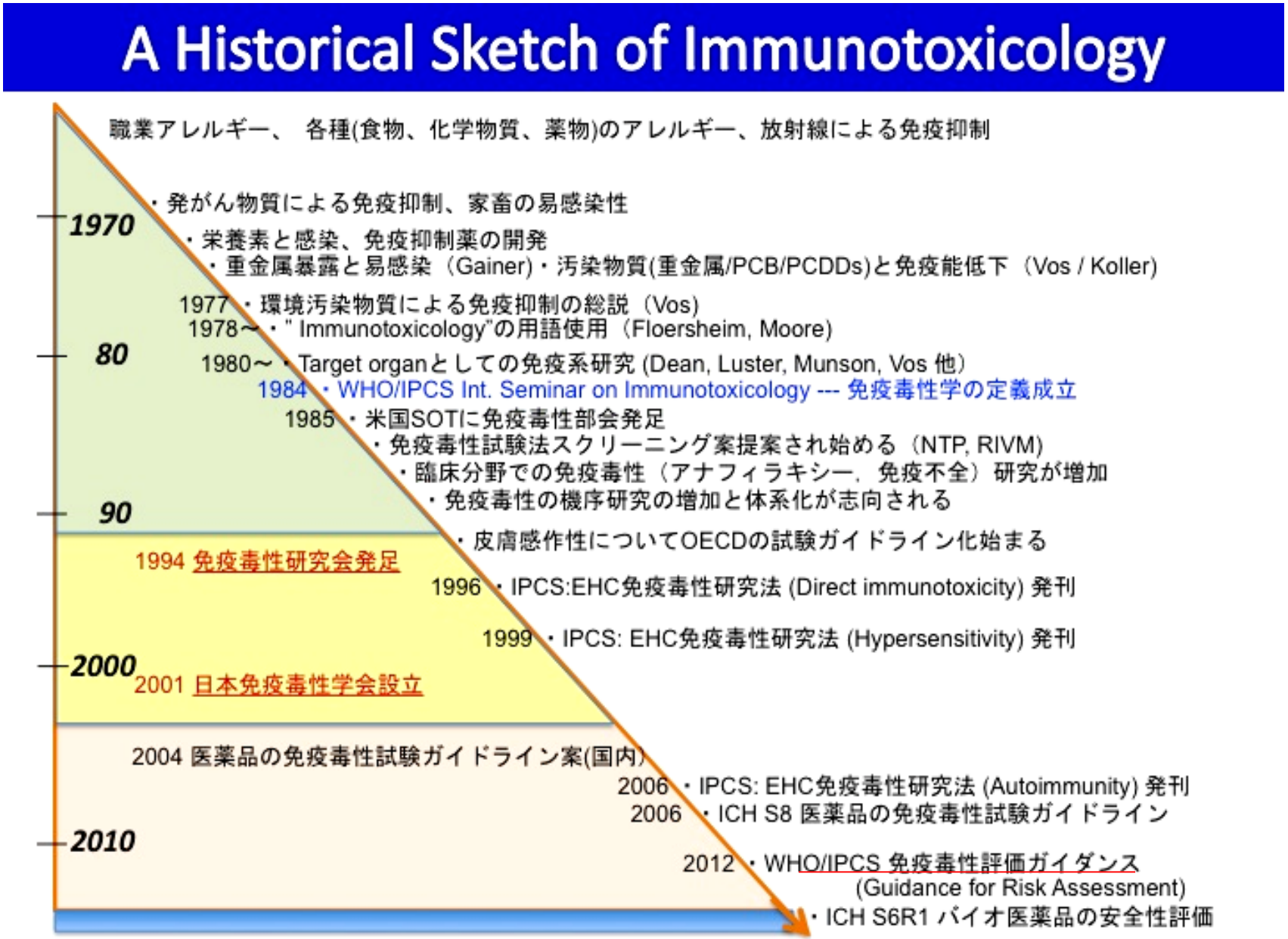
免疫毒性研究の流れ


A Historical Sketch of Immunotoxicology
職業アレルギー、 各種(食物、化学物質、薬物)のアレルギー、放射線による免疫抑制
・発がん物質による免疫抑制、家畜の易感染性
1970
・栄養素と感染、免疫抑制薬の開発
・重金属暴露と易感染（Gainer)・汚染物質(重金属/PCB/PCDDs)と免疫能低下（Vos / Koller)
1977 ・環境汚染物質による免疫抑制の総説（Vos)
1978～・" Immunotoxicology"の用語使用（Floersheim, Moore)
80
1980～・Target organとしての免疫系研究 (Dean, Luster, Munson, Vos 他)
1984 ・WHO/IPCS Int. Seminar on Immunotoxicology --- 免疫毒性学の定義成立
1985 ・米国SOTに免疫毒性部会発足
・免疫毒性試験法スクリーニング案提案され始める（NTP, RIVM)
・臨床分野での免疫毒性（アナフィラキシー，免疫不全）研究が増加
・免疫毒性の機序研究の増加と体系化が志向される
90
・皮膚感作性についてOECDの試験ガイドライン化始まる
1994 免疫毒性研究会発足
1996 ・IPCS:EHC免疫毒性研究法 (Direct immunotoxicity) 発刊
1999 ・IPCS: EHC免疫毒性研究法 (Hypersensitivity) 発刊
2000
2001 日本免疫毒性学会設立
2004 医薬品の免疫毒性試験ガイドライン案(国内)
2006 ・IPCS: EHC免疫毒性研究法 (Autoimmunity) 発刊
2006 ・ICH S8 医薬品の免疫毒性試験ガイドライン
2010
2012 ・WHO/IPCS 免疫毒性評価ガイダンス
(Guidance for Risk Assessment)
・ICH S6R1 バイオ医薬品の安全性評価

Immunotoxicity に関する既存のドキュメント

・**WHO/ IPCS** (International Programme on Chemical Safety)
：EHC (Environmental Health Criterium Documents)
(1) principles and methods for assessing direct immunotoxicity associated with exposure to chemicals (#180):1996
(2) principles and methods for assessing allergic hypersensitization associated with exposure to chemicals (#212): 1999
(3) principles and methods for assessing autoimmunity associated with exposure to chemicals (#236):2006

・**ICH** (The International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use)
・Immunotoxicology studies for human phaermaceuticals S8, p1-11 (2005) (http://www.ich.org//fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S8/Step4/S8_Guideline.pdf)

・**OECD**（化学物質の試験に関するガイドライン）
(1) Skin Sensitisation (TG406) guinea pig maximization test (1992)
(2) Skin Sensitisation: Local lymph node assay (TG429) (2002)
(3) Skin Sensitization: Local Lymph Node Assay: DA (TG 442A) ('2010)
(4) Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA (TG 442B) (2010)

免疫毒性リスク評価ガイダンス作成に至る準備

・2008.2.28-29: WHO/IPCS Scoping meeting for the development of guidance
(ガイダンスのスコープに関する会合(オランダRIVMにて))

・2009.4.27-29: WHO/IPCS Immunotoxicity drafting group meeting for public review
:(public reviewに向けたドラフト作成グループによる会合(オランダRIVMにて))

・2010.11.15- 2011.1.31 :draft guidance document was released on the Internet for public and peer review

・2011.10. 3-4: WHO/IPCS International Workshop on Immunotoxicity risk assessment for chemicals

・2011.10.5: Drafting group meeting
(ガイダンス及びcase study の最終化(オランダRIVMにて))

・2012.3: WHO/IPCS released a harmonized guidance (http://who.int/ipcs/method/harmonization/areas/guidance_immunotoxicity.pdf).

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WHO/IPCS 化学物質免疫毒性リスク評価ガイダンス
(Guidance for immunotoxicity risk assessment for Chemicals)

目的 (WHO/IPCS harmonization project)

- Increasing understanding and agreement on basic risk assessment principles
  リスク(毒性)評価の基本原理の理解と同意を得ること。
- Developing international guidance documents on specific issues
  特定領域における国際的ガイダンス文書の作成
- Enhancing the utility of risk assessments globally
  リスク評価手法の国際的な利用促進

目的 (免疫毒性ガイダンス)

・化学物質に対する免疫毒性を、国際的に同意された方法を用いて評価し、これらの評価が適切なリスク管理に用いられることをめざす。

・ 免疫毒性専門家よりなるワーキンググループを設置し、リスク評価を行う際の範例を示す作業を行う。

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概要

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2章：背景
3章：免疫毒性リスク評価のフレームワーク
4章：免疫抑制
5章：免疫促進
6章：感作性とアレルギー反応
7章：自己免疫誘発性

事例研究 (Case-study)のためのモデル化合物

鉛:4章のimmunosuppressionの事例

ヘキサクロルベンゼン(HCB):5章のimmuno-stimulationの事例

ハロゲン化プラチナ:6章のsensitizationの事例

芳香剤Citral:6章の(skin) sensitizationの事例

水銀:7章のautoimmunityの事例

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1章:序論

（目的）This harmonization project document provides guidance for immunotoxicity risk assessment for chemicals.

It encompasses studies of various immune pathologies, including allergy, immune dysregulation (suppression or enhancement), autoimmunity and chronic inflammation.

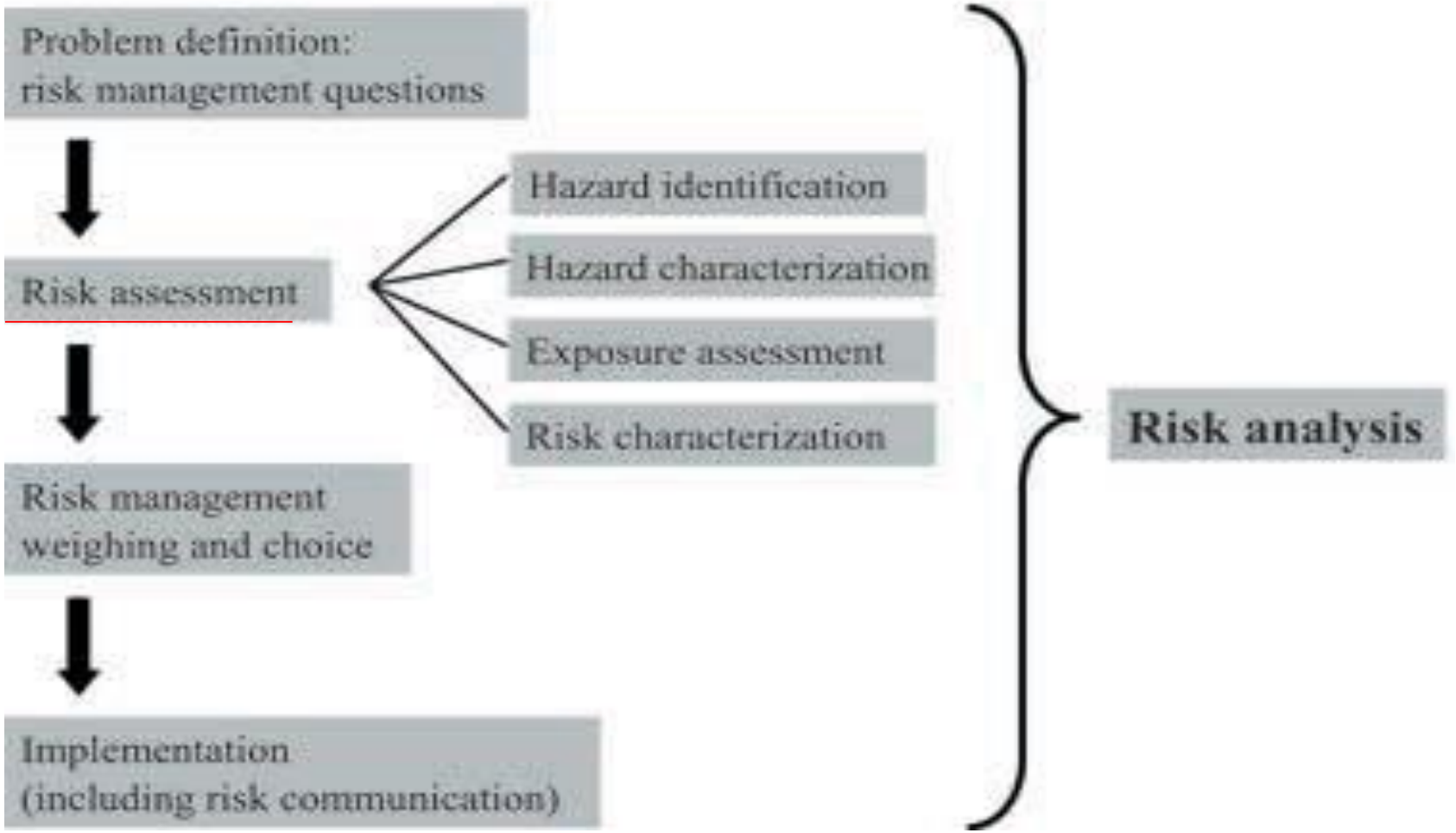
(範疇） The risk assessment process consists of four main steps: hazard identification, hazard characterization, exposure assessment and risk characterization.

(内容）chapter 2; outlines of special features of immune system

chapter 3: a framework for immunotoxicity risk assessment for chemicals, comprising entry points that help to determine whether or not immunotoxicity needs to be considered and what type of immuntoxicity needs to be evaluated.

chapter 4-7: review and risk assessment guidance for the different type of immunotoxicity, addressing immunosuppression (chapter 4), immunostimulation (chapter 5), sensitization and allergic response (chapter 6), and autoimmunity and auto-immune disease (chapter 7)

Risk analysis: the process of setting objectives, identifying hazards, assessing risks and management options, weighing these, and prioritizing, choosing and implementing risk management measures



Risk assessment (リスク評価) と Risk analysis ((リスク分析)の関係

2章:背景 (その1)

・Imunotoxicity risk assessment of chemicals is an evaluation of the potential for unintended effects of chemical exposure on the immune system

・These effects manifest as four principal types of immunotoxicity: immunosuppression, immunostimulation, sensitization, autoimmunity.

・It is well established that xenobiotic-related immunosuppression can lead to reduced resistance to infections and certain neoplastic diseases.

・Exposure to xenobiotics has been shown to be associated with development on worsening of autoimmune disease.

・It is also well established, that xenobiotics can elicit hypersensitivity responses directly as an allergen, or they can enhance the induction or severity of allergic sensitization to allergens such as pollen or house dust mites.

2章:背景 (その 2)

The guidance states that immunotoxicity risk assessment should be performed according to the same principal approaches as applied in risk assessment for other (thresholded) toxicological end-points, but the immune system manifests many special aspects that need specific consideration in risk assessment.

3章：免疫毒性リスク評価のフレームワーク

Hazard identification and hazard characterization

- Clinical and epidemiological data
- Animal data: dose response relationships and thresholds, exposure duration, species and strain consideration, age at initial exposure, gender, route of exposure, local versus systemic effects, irreversibility of effects, acute versus chronic exposure

Exposure assessment: severity and persistence, exposure timining and susceptibility, route of exposure and local immunity, toxicokinetic considerations

Risk characterization: Ideally, a quantitative risk assessment is performed by quantitative dose-response assessment and exposure assessment , but a qualitative risk assessment may still be possible.

- Until now, most immunotoxicity assessments are done with animal experiments, but more and more, emphasis is placed on the human.
- The guidance recommends that a weight of evidence approach is most suited for the purpose of risk assessment of immunotoxicity. This approach should include clinical and epidemiological information, as well as information from animal testing and other Information.

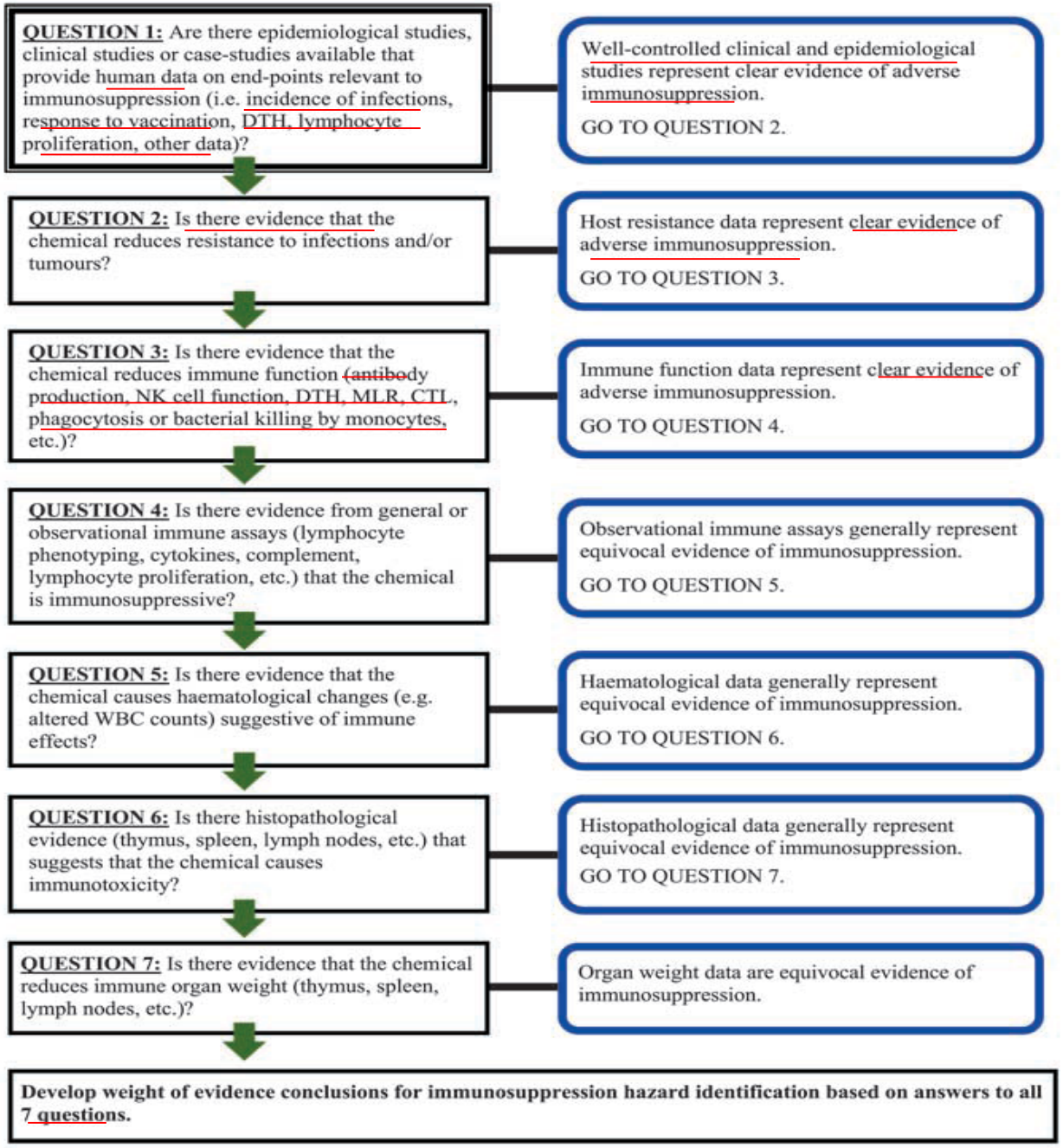
4章：Assessment of immunosuppression (免疫抑制）

Hazard identification: Functional assays measure the response of the immune system to a challenge at the cellular or whole-animal level. This assay type provides the best evidence of immune system health by mimicking host responses that reduce the risk of infection (e.g.producing antibodies in response to immunization). Certain guidelines that include screening for potential immunotoxicants (e.g. OECD Test Guideline 407, WHO/IPCS's EHC 180, ICH S8 protocols, the EU's REACH) rely on changes in observational end-points to trigger assessment of immune function. Fruthermore, human data should be used whenever available and should take precedence over extrapolation from laboratory animal data, provided that equivalent endpoints are compared and the data are of sufficient quality and reliability.

Hazard identification for immunosuppression should result in weight of evidence conclusions based on the available human and laboratory animal data for a given chemical. The following outline presents a structured approach to organizing the available data for developing weight of evidence conclusions in the assessment of immunosuppression hazard identification through seven questions asking the risk assessor to evaluate the available data from the strongest and most predictive data (human data) through the least predictive (immune organ weight), that is; 1) *Human data*, 2) *Host resistance (laboratory animal data)*, 3)*Immune function (laboratory animal data)*, 4) *General immune assays (laboratory animal data)*, 5)*Haematology (laboratory animal data), 6) Histopathology (laboratory animal data)*, 7)*Organ weight (laboratory animal data).*

Hazard characterization: Even moderate suppression in humans may decrease responses to immunization and increase susceptibility to infection and certain types of cancer.

Dose–response relationships and thresholds:A dose–response relationship is a necessary criterion in demonstrating chemical immunosuppression. The critical effects are then used for the development of POD(s)(point of departure) from which health-based guidance values or reference values (ADI/TDI or RfD/RfC) can be calculated by dividing the POD(s) by the total uncertainty factor.



Weight of evidence approach for assessment of immunosuppression.

The figure presents a structured approach for organizing all of the available data for developing weight of evidence conclusions for immunosuppression hazard identification. It presents a summary of categorical data binnig, from the most to least predictive, rather than a decision-tree.
CTL, cytotoxic T lymphocyte; MLR, mixed leukocyte reaction

(質問7項目）

Case-study 1:Assessment of immunosuppression caused by lead exposure

(1) Application of the weight of evidence approach:

1) Human data (Yes) : It does appear that PMNLs are one of the targets of lead's toxicity. One study assessed exposed to lead (Queiroz et al., 1994). Phagocytosis of both antigens and phagocytic splenic function were normal in all workers; however, the lytic activity of *C. albicans* was impaired. The average BLL (Blood lead level) of the 33 workers examined was 43.2 µg/dl. Application of the uncertainty factor (300) to the BLL obtained from the study (i.e. the POD) results in a BLL of 0.144 µg/dl (i.e. 43.2/300) as the AEL (acceptable exposure level).

2) Host resistance (laboratory animal data) (Yes): In the Fernandez-Cabezudo et al. (2007) study, C3H/HeN mice were exposed to lead acetate in the drinking-water and examined the susceptibility to *Salmonella* infection. Lead exposure increased susceptibility to *Salmonella* infection in mice. The LOAEL for lead acetate in this study was 1036 mg/l, with a corresponding BLL of 20.5 µg/dl. Application of the uncertainty factor (3000) to the BLL obtained from the LOAEL (i.e. the POD) results in a BLL of 0.0068 µg/dl (i.e. 20.5/3000) as the AEL.

3)Immune function (laboratory animal data) (Yes): The data on DTH suppression in BALB/c mice may be the most complete and reproducible data set on lead. As DTH response was suppressed at 512 mg/l , the 512 mg/l dose with the corresponding BLL of 87 µg/dl was used as a LOAEL. When one applies this uncertainty factor(3000) to the BLL obtained from the LOAEL (i.e. the POD), the AEL is 0.029 µg/dl (i.e. 87/3000).

4) General immune assays (laboratory animal data) (Yes): Lead exposure in animals causes a shift in immune cells to immature cell types (progenitor cells) (Burchiel et al., 1987).

5)Haematology (laboratory animal data) (Yes): Very few immunotoxicological studies reported significant haematological effects from lead exposure.

6) Histopathology (laboratory animal data) (No):Faith et al. (1979) reported that there were no histopathological differences between the organs in the control and exposed groups.

7)Organ weight (laboratory animal data) (Yes):As immune organ weight data are limited in animals and contradictory for the spleen, these data are equivocal for assessment of immunosupression.

(2) Conclusion: The data in experimental animals and humans, although variable, suggest that lead suppresses defence mechanisms.

5章：Assessment of immunostimulation（免疫促進）

Hazard identification:

This chapter will examine the evidence to support the hypothesis that unintended stimulation of either the innate or adaptive immune response should be considered as an adverse effect and taken into account in a weight of evidence approach to risk assessment.

Hazard identification for immunostimulation should result in weight of evidence conclusions based on the available human and laboratory animal data for a given chemical.

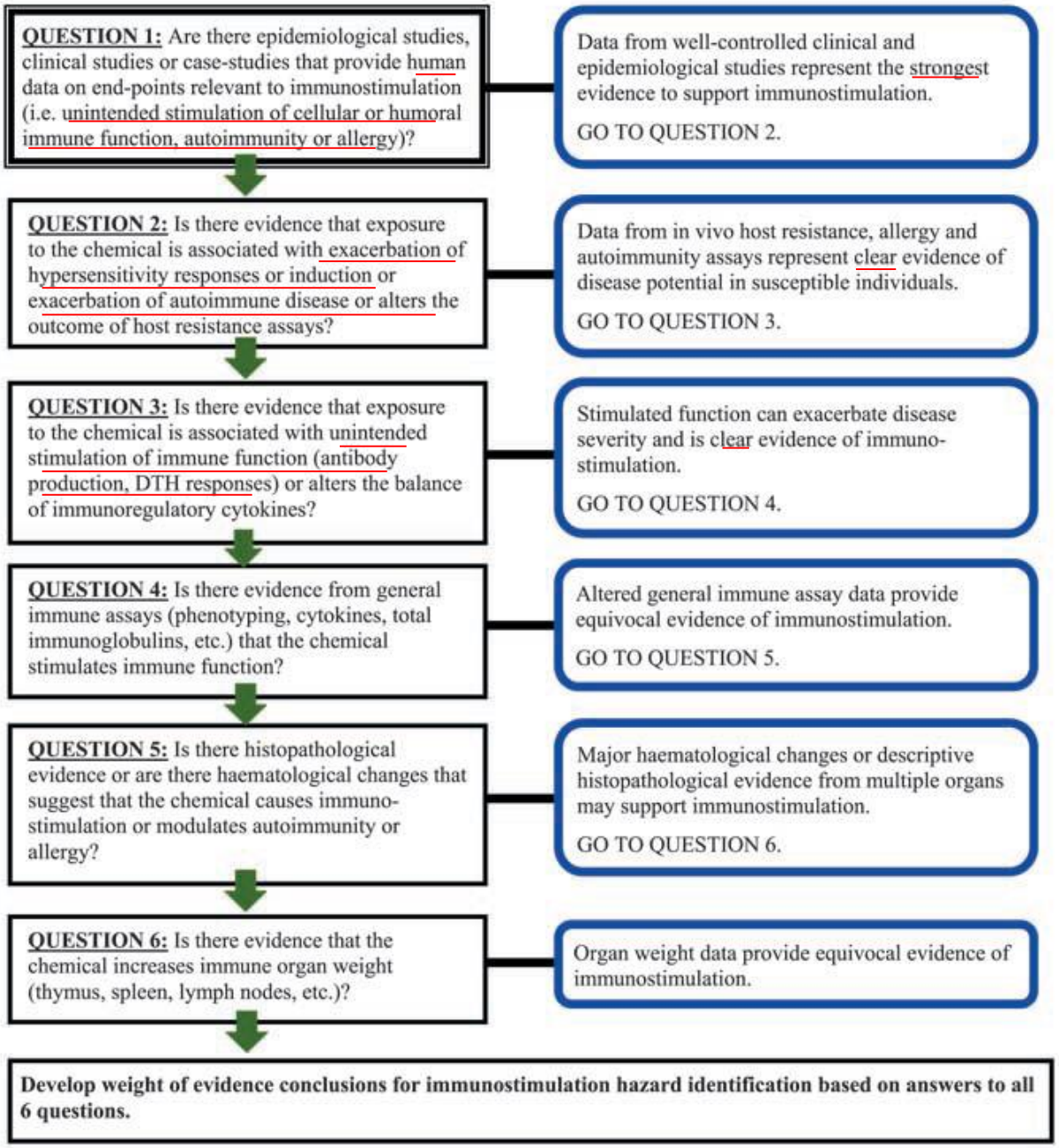
Six questions are arranged to evaluate the available data from the strongest and most predictive data (human data) through the least predictive (immune organ weight) as follows: (1) Human data, (2) *Allergic, autoimmune or infectious disease (laboratory animal data)*, (3) *Immune function (laboratory animal data)*, (4) *General immune assays (laboratory animal data)*, (5) *Histopathology and haematology (laboratory animal data)*, (6) *Organ weight (laboratory animal data).*

Hazard characterization:

Because inflammation is a normal response to toxicity, the possibility exists that toxic exposures can synergistically or additively increase inflammatory responses to infectious or allergen challenge. In animal models, several types of chemical exposure, most notably to dioxin, have been shown to increase pulmonary damage caused by the immune response to influenza infection. Similarly, exposure to air pollutants has been shown to exacerbate respiratory responses to allergen challenge in rodent and human studies, and air pollutants act as adjuvants to promote allergic sensitization

Risk characterization:

As is true for all forms of immunotoxicity, ideally, a quantitative risk assessment for immunostimulation associated with chemical exposure is performed. In the case where the available data do not allow for this, a qualitative risk assessment may be possible.



Schematic for organizing all available data for a weight of evidence approach for assessment of immunostimulation.

The figure presents a summary of categorical data binning,from the most to least predictive, rather than a decision-tree

Case-study 2:Assessment of immunostimulation induced by hexachlorobenzene(HCB)

(1) Application of the weight of evidence approach:

1) **Human data** (Yes): Human data provide limited evidence for HCB-induced immune effects. Some effects, such as the enlarged lymph nodes and the development of arthritis identified in the Turkish incident and the observed increase in serum IgM and IgG levels in the Brazilian plant workers, point towards immunostimulation caused by HCB.
2) **Allergic, autoimmune or infectious disease (laboratory animal data)** (Yes**):** The highest dose of HCB (22.5mg/kg bw per day) increased the severity of EAE(experimental allergic encephalomyelitis).
3) **Immune function (laboratory animal data)** (Yes**):** HCB increased humoral responses to tetanus toxoid and DTH in the offspring of rats after perinatal exposure. the lowest dose was 0.2mg/kg body weight per day.
4) **General immune assays (laboratory animal data)** (Yes): In rats, dietary exposure to HCB stimulates responses in general immune assays.
5) **Histopathology and haematology** (Yes) Oral HCB exposure induced histo-pathological and haematological changes in rats,monkeys and dogs suggestive of immunotoxicity.
6) **Organ weight (laboratory animal data** (Yes): In rats, oral exposure to HCB dose-dependently increased the weight of the spleen and lymph nodes, but did not affect the weight of the thymus.

(2) Conclusion: The weight of evidence approach determined that HCB can be considered as an immunostimulatory chemical. The AEL for these immune effects Is much lower for developmental exposure than for adult exposure.

6章：Assessment of sensitization and allergic response（感作性)

Hazard identification: it is clear that risk assessment for chemically induced hypersensitivity has two components: 1) the likelihood that a chemical will induce sensitization in a previously nonsensitized individual and 2) the likelihood that a chemical will provoke an allergic reaction in those who are already sensitized.

In this chapter, guidance will be developed for the conduct of risk assessments for both the induction and elicitation of skin allergy, respiratory allergy and oral (systemic) allergy. The most progress in this regard has been made with allergic contact dermatitis; tools for dealing with respiratory allergy are more limited, and systemic (oral) allergy has received the least attention to date. Three decision-trees (Figures 6.2A, 6.2B and 6.2C）have been developed as a guide through the process of assessing sensitization and allergy caused by exposure to chemical substances via the dermal, inhalation and systemic routes.

Depending on the data situation and on the scope of the risk assessment, it may be advisable to address all routes of exposure, that is, to use all three decision-trees, or it may be sufficient to use only one decision-tree, if the relevant sensitization route has already been clearly identified.

Hazard characterization: Many predictive test methods serve simply to identify the inherent potential of a chemical to induce allergy but provide no indication of the potency with which it will do so. One problemis that some methods do not incorporate a dose–response analysis or identification of a threshold (or NOEL)..

Dose–response relationships and thresholds: In a number of studies, human NOELs and BMDs were compared with LLNA thresholds (EC3 values), and it was found that the average ratio of both values is close to 1, indicating that area doses are directly comparable between mice and humans.. .

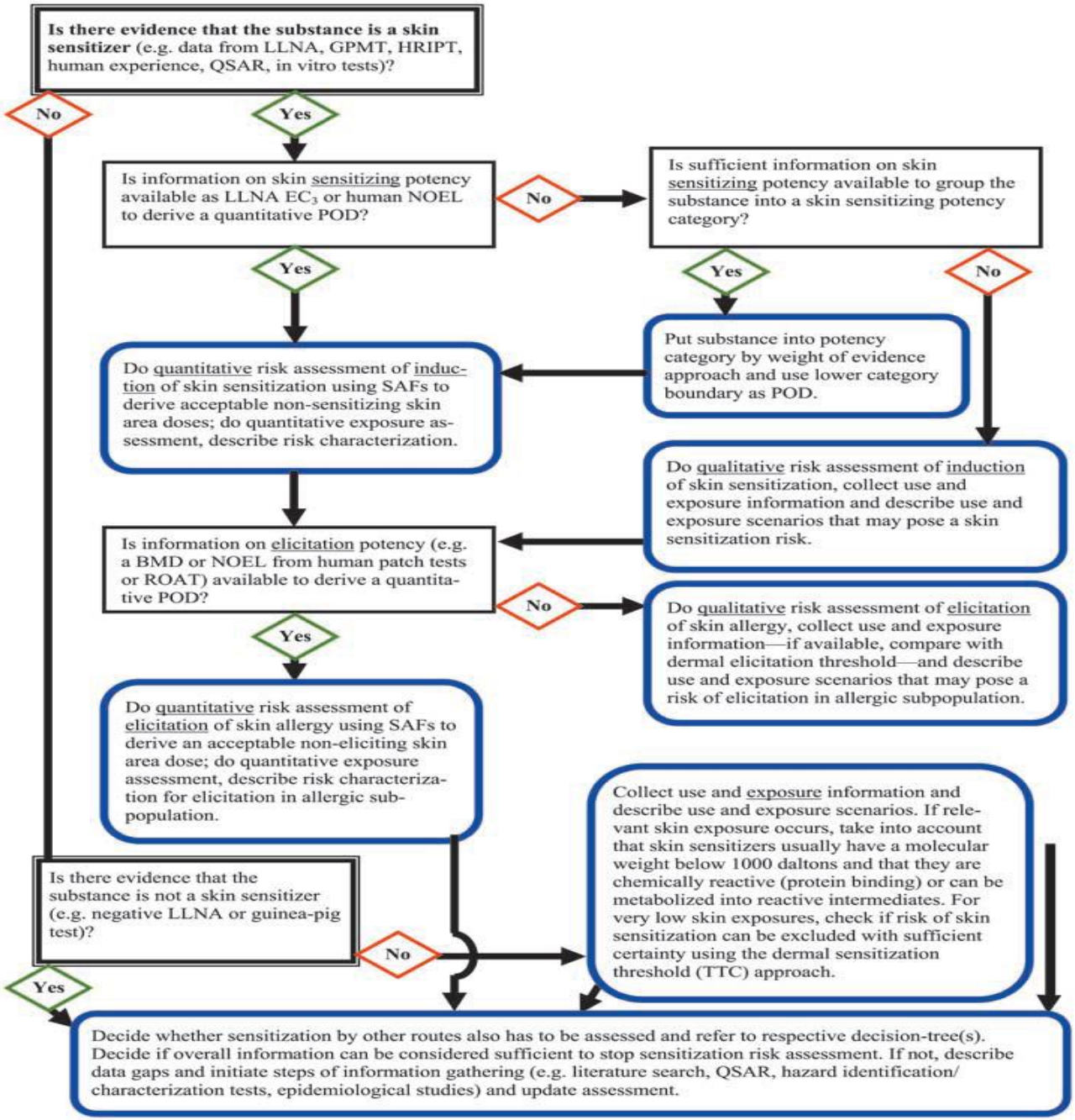


Figure 6.2A: Decision-tree for the assessment of sensitization and allergic response: skin sensitization.

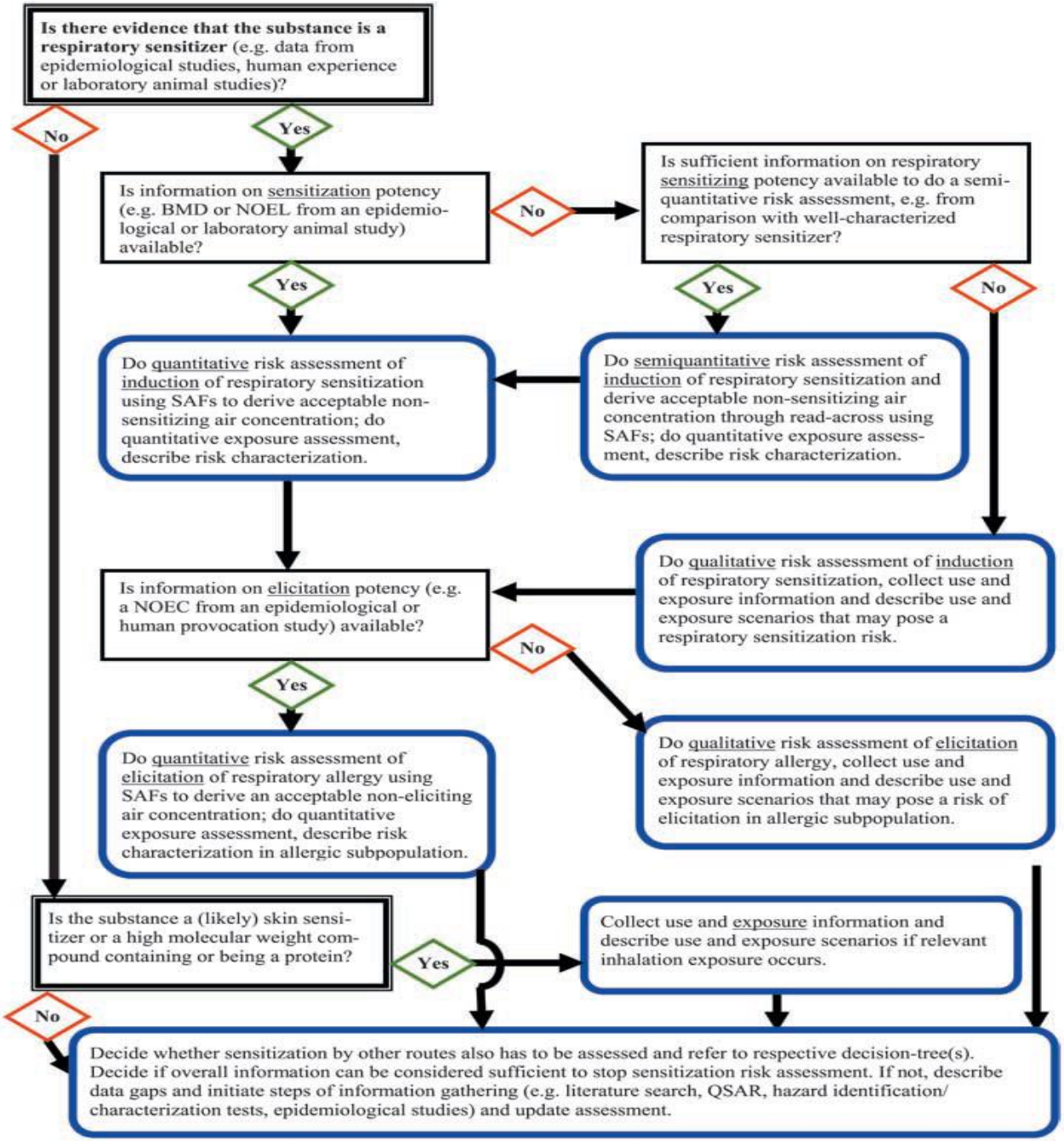


Figure 6.2B Decision-tree for the assessment of sensitization and allergic response: respiratory sensitization.

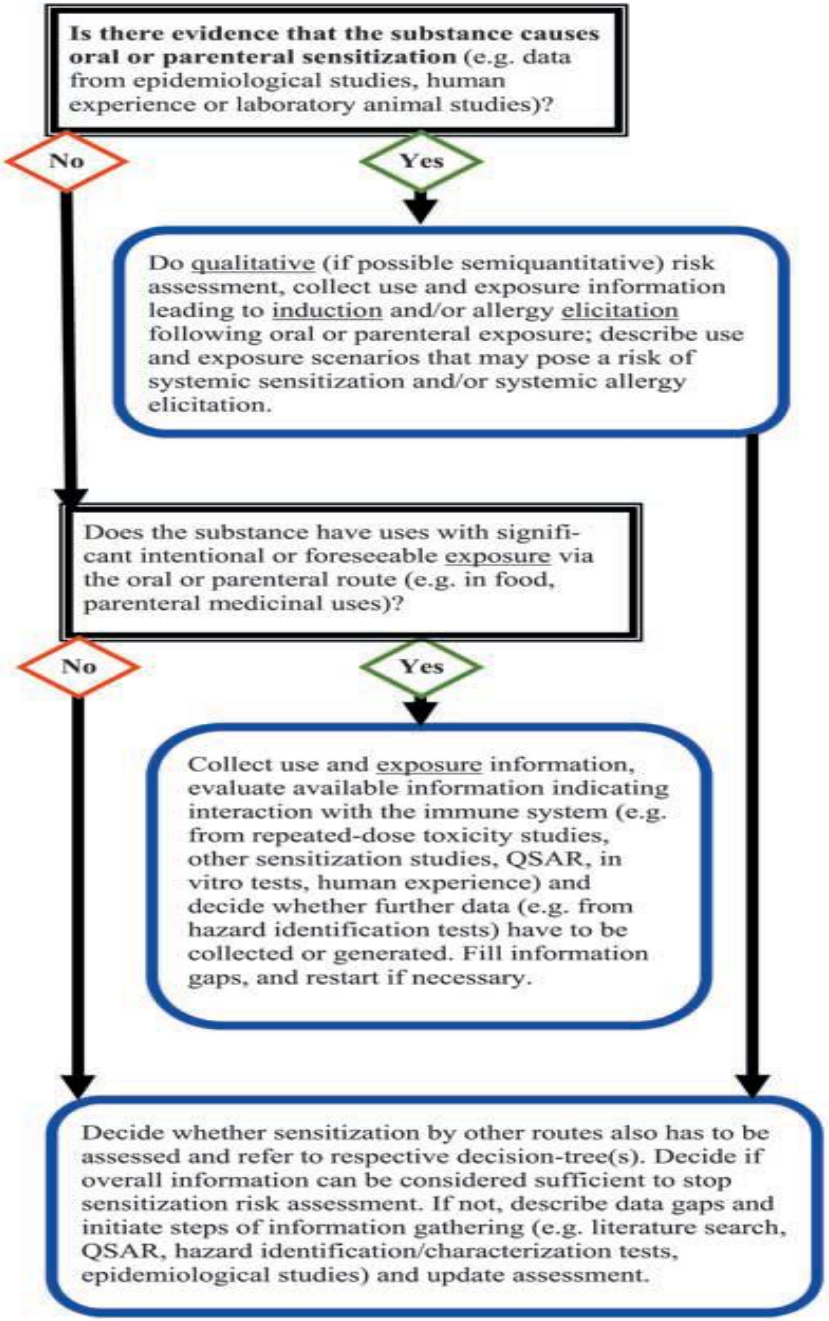


Figure 6.2C
Decision-tree for the assessment of sensitization and allergic response: systemic sensitization.

Case-study 4:Assessment of skin sensitization to citral

Application of the weight of evidence approach (Fig.6.2A):

1) **Is there evidence that the substance is a skin sensitizer(e.g. data from LLNA, GPMT, HRIPT, human experience, QSAR, in vitro test)?** (Yes): Citral has been tested extensively for skin sensitization in guinea-pigs, mice and humans, and in all species, citral has tested positive for skin sensitization. Citral was found to be sensitizing in the guinea-pig at 1% in petrolatum

2) **Is information on skin sensitizing potency available as LLNA EC3 or human NOEL to derive a quantitative POD?** (Yes): The derived human NOEL of 1400 µg/cm2 from the HRIPT data is well supported by the vehicle-weighted mean LLNA EC3 of 1609 µg/cm2 and was therefore set as the POD for the assessment of induction of skin sensitization (also referred to as no expected sensitization. Based on the LLNA EC value of 5.6% (Api et al., 2008) or 5.7% (Loveless et al., 2010), citral can be classified in the weak to moderate potency range of skin sensitizers (ECETOC,2003).

3) **Is information on elicitation potency (e.g. a BMD or NOEL from human patch tests or ROAT) availabe to derive a quantitative POD?** (No);There are no quantitative data on the elicitation potency of citral.

Conclusion: Citral was selected because it represents an example of the group of fragrance ingredients that are well established as skin sensitizers. Possible measures could include, for example, labels and use instructions on consumer products, bans or concentration limits for certain uses, and personal protection measures at the workplace.

7章：Assessment of autoimmunity and autoimmune disesase（自己免疫）

Hazard identification:

Autoimmunity and autoimmune diseases result from immune responses against selfmolecules. The immunological effectors and mechanisms involved in autoimmune reactions are the same as 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.

Five questions are arranged to evaluate the available data from the strongest and most predictive data (human data) through the least predictive (immune organ weight) as follows:

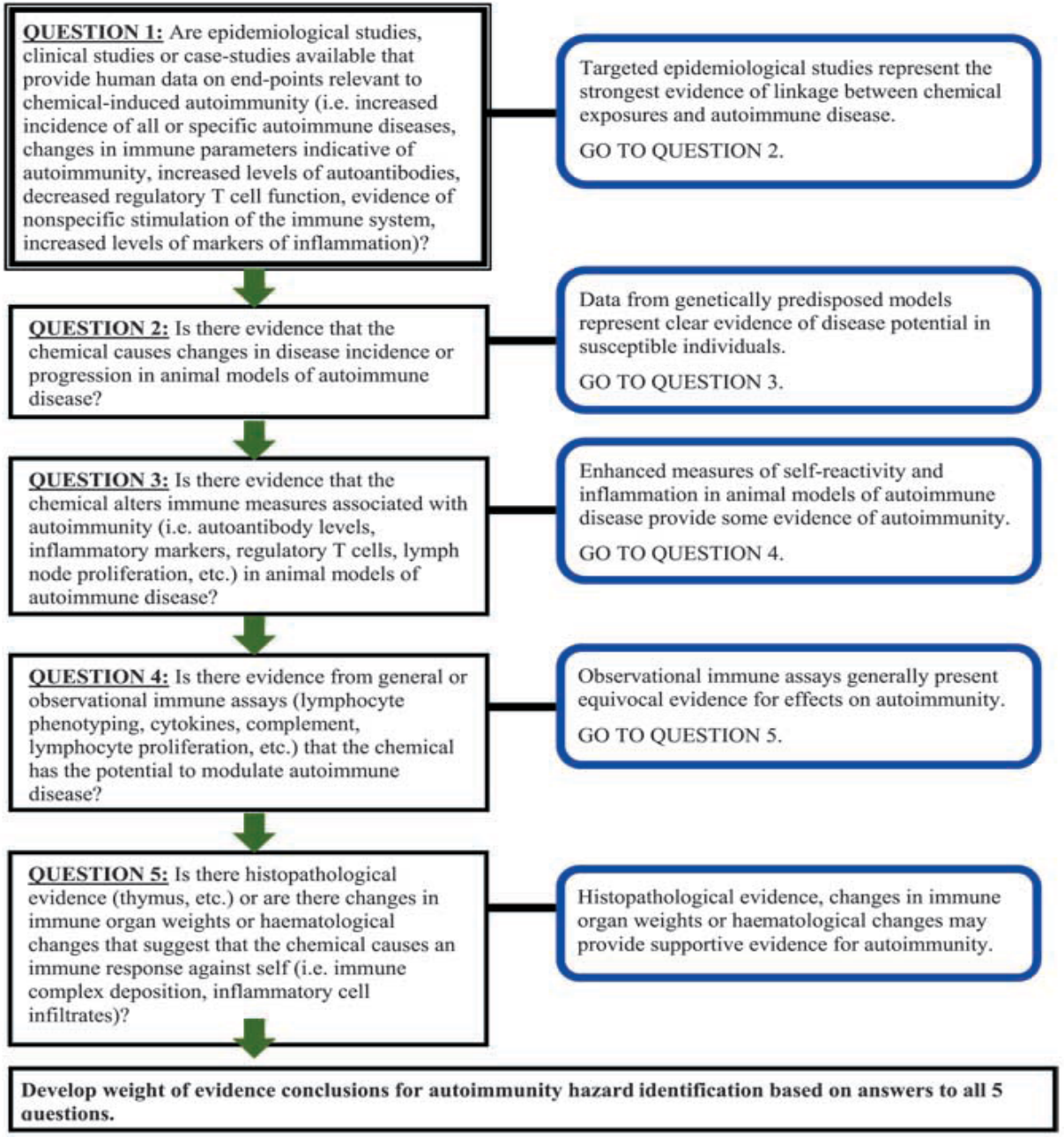
(1) Human data, (2) *Modulation of disease incidence or progression (laboratory animal data)*:, (3) *Immune function (laboratory animal data)*, (4) *General immune assays (laboratory animal data)*, (5) *Histopathology and haematology (laboratory animal data).*

Hazard characterization:

A basic understanding of the typical methodologies used to evaluate the induction or exacerbation of autoimmunity in animal models is necessary to evaluate the database of studies for hazard characterization of a given chemical as the first step in risk assessment. Detailed discussions of end-points and methods utilized in characterizing autoimmunity are provided in EHC 236:.

Risk characterization:

As is true for all forms of immunotoxicity, ideally, a quantitative risk assessment is performed for autoimmunity associated with chemical exposure. In the case where the available data do not allow for this, a qualitative risk assessment may be possible.



Schematic for organizing all available data for a weight of evidence approach for assessment of chemical-induced auto-immunity.

The figure presents a summary of categorical data binning, from the most to least predictive, rather than a decision tree.

Case-study 6:Assessment of autoimmunity-stimulating effect of trichloroethylene (TCE)

(1) Application of the weight of evidence approach:

1)Human data (Yes): TCE induces clinical disorders similar to idiosyncratic drug hypersensitivity reactions, as well as clinical disorders that may be linked to autoimmunity, with the strongest data on autoimmunity in humans supporting an association between TCE and systemic sclerosis (scleroderma) (NRC, 2006;Cooper et al., 2009).

2) Modulation of disease incidence or progression (laboratory animal data) (Yes): Most (Khan et al., 1995; Griffin et al., 2000a,b,c; Blossom et al., 2006, 2007, 2008; Gilbert et al., 2006, 2009; Blossom & Doss, 2007), but not all (Peden-Adams et al., 2008;Keil et al., 2009), studies using autoimmune disease–prone strains of mice (MRL+/+ mice) suggest that TCE promotes pathogenesis and progression of autoimmune disease in several mouse models of autoimmune disease and induces biomarkers of autoimmune disease in wild-type mice. However, studies to date have not demonstrated that TCE induces autoimmune disease.

3) Immune function (laboratory animal data) (Yes**):** There are a number of studies that demonstrate TCE modulation of immune measures associated with autoimmunity in mouse models of autoimmune disease.

4) General immune assays (laboratory animal data) (Yes): TCE as well as its metabolites TCAH and TCA have been demonstrated to activate CD4+ T cells in autoimmune disease–prone MRL+/+ mice.

5)Histopathology and haematology (Yes):The main histopathological evidence of TCE-associated autoimmunity is from studies reporting leukocyte infiltration.

(2) Conclusion: A risk assessment for an autoimmune disease–inducing or auto- immune disease–stimulating property of TCE is indicated. The case-study also encountered limitations of evaluating human data.

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III. まとめ

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(1) 化学物質の免疫毒性に関してのリスク評価を目的とした国際的ガイダンスが作成された。このガイダンスは、ヒトが化学物質と免疫毒性を与える濃度で摂取することを防ぎ、公共の健康を守ることを意図したものである。
(2) さらに、免疫毒性評価の国際的なハーモナイゼーションを容易にし、透明性を確保し、行われた免疫毒性評価の相互の理解と共有を図り、労力の軽減を図ることも意図されている。
(3) このガイダンスでは、6種の化合物によるケーススタディー結果が報告され、化学物質の持つ免疫毒性をどのように評価するかの例が示されている。

免疫毒性ガイダンスの今後について

毒性学の分野は、用いる手法、メカニズム解析共に急速に進歩している。
また、一方で、動物実験を減らすようにとの動物愛護の観点からの要望もあり、In vitro代替法試験系の開発やオミックス技術の発展が目覚ましい。
この免疫毒性ガイドラインも、将来的な毒性評価技術の発展に伴って、見直されるものと思われる。