Safety evaluation strategy of non-intentionally added substances (NIAS)

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Outline

- Safety evaluation of FCM
- Definition of non-intentionally added substances (NIAS)
- NIAS safety evaluation strategies
- Novel safety strategy applied to UNKNOWN substances; an example on a paper FCM
- Challenges for applying TTC to unknowns
- Outlook
Safety evaluation of FCM: intentionally added substances (IAS)

› Traditionally, check composition starting materials FCM (IAS) + compliance specific migration limit (SML) substances.

› Examples of IAS;
  › Monomers
  › Pre-polymers
  › Base materials
  › Antioxidants
  › Polymer production aids
  › Catalysts
  › …

No full safety evaluation!
Safety evaluation of FCM; IAS + NIAS

Plastics legislation requires IAS and NIAS (non-intentionally added substances) to be assessed.

article 3 of the Plastic Regulation (EU) No 10/2011 ‘non-intentionally added substance (NIAS)’; an impurity in the substances used or a reaction intermediate formed during the production process or a decomposition or reaction product.
(18) Substances used in the manufacture of plastic materials or articles may contain impurities originating from their manufacturing or extraction process. These impurities are non-intentionally added together with the substance in the manufacture of the plastic material (non-intentionally added substance – NIAS). As far as they are relevant for the risk assessment the main impurities of a substance should be considered and if necessary be included in the specifications of a substance. However it is not possible to list and consider all impurities in the authorisation. Therefore they may be present in the material or article but not included in the Union list.
Definition NIAS; recitals Plastic Regulation (EU) No 10/2011

(20) During the manufacture and use of plastic materials and articles reaction and degradation products can be formed. These reaction and degradation products are non-intentionally present in the plastic material (NIAS). As far as they are relevant for the risk assessment the main reaction and degradation products of the intended application of a substance should be considered and included in the restrictions of the substance. However it is not possible to list and consider all reaction and degradation products in the authorisation. Therefore they should not be listed as single entries in the Union list. Any potential health risk in the final material or article arising from reaction and degradation products should be assessed by the manufacturer in accordance with internationally recognised scientific principles on risk assessment.
Examples NIAS

- Example of NIAS;
  - Oxidation product e.g. oxidized irgafos 168
  - Oligomers?
  - Impurities
  - Contaminants of processing/transportation
  - Starting substances to make e.g. bisphenol-A (acetone, phenol)
  - ...

- Does it matter that definition of NIAS can be interpreted differently?
  - **No** Final article should not endanger human health
  - **Yes** - If detected peak is NIAS it may be present in article
    - If detected peak is IAS it should be petitioned.

→ task ILSI working group on NIAS
How to do your safety evaluation for NIAS?

› Applying traditional approach requires that EVERYTHING that migrates is identified and risk assessed (full composition, IAS+NIAS).

› Obtain information on NIAS throughout the value chain?

› Feasible?

› What to do if full identification is not possible…?
Introduction

Most relevant data to start with:
- Identity (purity, size, shape, surface area, etc…)
- Physico-chemical properties (chemical reactivity, (photo)catalytic reactivity, surface charge, etc…)

Changes in either identity and/or physico-chemical properties may introduce specific hazards.

Current approach

- Focus on full identification
  - Identify & quantify all components
  - Hazard & risk assessment for each individual component
  - Low detection levels required
  - CRM substances < 10 ppb not covered

Innovation

- Focus on toxicological relevance
  - Threshold for identification uprated from 10 ppb to 90 µg exposure per day
  - Hazard & risk assessment only for substances above exposure threshold
  - High risk compounds covered by targeted analyses

Exposure threshold

10 ppb
Innovation concept for safety evaluation of FCM

Generic health limit based on the TTC decision tree (Kroes et al 2004)

Basic principle in toxicology:

"Everything is poison and there is poison in everything. It is the dose that makes a thing a poison."

Risk = Exposure x Hazard
STEP 1
Translate response into intake and identify peaks corresponding with intakes of more than 90 µg/p/d

STEP 2, exclude:
proteins (or assess safety)
non-essential/heavy metals
metal containing compounds
dioxin-like chemicals
high potent genotoxic compounds
organophosphates

STEP 3, exclude:
(structural alerts for) genotoxicity

STEP 4
Identify and assess compounds with intakes >90 µg/p/d and non-excluded compounds

STEP 5, assess allergenicity

1 based on Cramer class III

Rennen et al. 2011
Step 1: Screening techniques

Combination of techniques covering broad spectrum of substances

- Headspace/SPME GC-MS  
  Volatile substances
- GC-FID/MS  
  Semi-volatile subst.  
  Medium polar/apolar subst.
- Derivatisation* GC-FID/MS  
  Non/semi-volatile subst.  
  Small polar/medium polar subst.
- LC-UV/light scattering/MS  
  Non volatile subst.  
  Polar – apolar subst.

*silylation makes non-volatile substances more volatile
Step 1: Example NIAS; semi volatiles (GC-MS)

90 µg daily intake (line to guide the eye)

Tenax
10 days 60 °C
GC-MS,
AT-5 column

Note;
check compliance IAS+ use chemical information to predict NIAS

10 ppb (line to guide the eye)
Step 1: Example NIAS; non-volatiles (LC-ELSD/UV)

- Tenax
- 10 days 60 °C
- H₂O → ACN,
  RP column

90 µg daily intake
Step 2: exclude known high toxic compounds and other TTC excluded classes

Not all classes relevant for FCM. Exclusion based on available information; expert judgment and/or targeted analysis

- Aflatoxins (relevant for material of biological origin) - ok
- Azoxy substances (not likely in FCM) - ok
- N-nitroso compounds (in rubber materials) - ok
- Steroids (negligible in FCM) - ok
- Dioxins (biological origin + chlorine containing FCM) - To do
- High MW substances e.g. polymers (in general not of tox relevance) - ok
- Non-essential metals (targeted analysis: ICP MS) - ok
- Proteins (screen for allergenicity, see step 5) - ok
- Organophosphates (targeted analysis; TTC of 18 µg/p/d) - ok

Excluded by analysis - ok
Excluded by expert judgment - ok
Step 2: Example NIAS; organophosphates (GC-NPD)

18 µg daily intake

NB - pesticides excluded with targeted LC-MS
  - LC-MS accurate mass to exclude P
Step 3: Exclude (structural alerts for) genotoxicity

Chemical analysis
Excluding genotoxicity by chemical analysis very difficult (21 structural alerts).

Bioassays
Conventional assays
AMES, MLA, CA not developed for complex matrices (higher concentration sensitivity required)

New developments; e.g. Bluescreen
- Luminescent assay (sensitive)
- Human cell line covering endpoints of Ames, micronucleus, mouse lymphoma assay and cytotoxicity
- \textit{GADD45a} gene
- Assay validated for pharmaceutical formulations
- High throughput! (96 well-format)
- Test protocol developed for complex matrices
Step 4: Evaluation of compounds above 90 µg daily exposure

- Identification and quantification of substances > 90µg daily exposure
- Determine substance specific threshold
  - Refinement Cramer classification using:
    - Cramer (1978)
    - open source applications such as Toxtree
- Substance specific toxicity data
  - retrieved from public literature
  - toxicity data from structural related substances (read-across).

Risk assessment; from ~60 substances (FOP) to ~10 substances (TTC)
Step 5: Assess allergenicity

- Proteins might give allergic responses in sensitive people and should therefore be evaluated.
- FCMs with ingredients of biological origin might contain proteins.
- If considered relevant screening for known allergens and specific risk assessment.
- Here assumed that allergens are not part of the FCM under investigation.
Challenges for applying TTC approach to unknowns?

- Combination toxicity – dose/concentration addition
  
  Current analytical approaches do not take this into account.
  
  However, at low exposure …

- Synergistic effects only when 2 or more compounds are above effect level (not likely at low TTC exposure)
- Cumulative effects is depending on potency, limited effect to be expected at low (90 µg/p/d) exposure. Eg.:
  - 21 or 26 organophosphates all at 90 µg/p/d => 360 or 250 µg/p/d when potency corrected for acute or chronic effects, respectively
  - 7 or 11 triazoles all at 90 µg/p/d => 240 or 300 µg/p/d when potency corrected for acute or chronic effects, respectively

Leeman et al. in preparation
Challenges for applying TTC approach to unknowns?

- Bio-accumulating substances

Log Po/w as ‘marker’ for accumulation

Three studies where no relation was found between log Po/w and NOAEL:

• Ravenzwaay (2011): 111 NOAELs from developmental rat studies (Log Po/w: -4.3 to 15; median 2.12)
• Ravenzwaay (2012): 104 NOAELs from developmental rabbit studies (Log Po/w: -13 to 15)
• Kalkhof (2012): 824 NOAELs from (28/90 day) repeated dose studies (Log Po/w: -2.76 to 7.1 [5th/95th Percentile]; median 2.36)
  - Relevance of accumulation at low exposure???
  (polyhalogenated and metals already excluded)
Challenges for applying TTC approach to unknowns?

Universal response detectors used?
- GC-FID/MS proven to give ‘uniform’ response for different analytes (factor ~6)

- Light scattering detectors (e.g. ELSD) for LC have fair uniformity (factor ~6) when operated with post-column addition of reverse gradient

Do you detect all substance in extracts?
No technique exists that can do so, however state-of-the-art used here.
Challenges for applying TTC approach to unknowns?

- TTC should not be used for mixtures containing unknown chemical structures?
  - Combination toxicity effects marginal?
  - Analytical screening as good as other approaches?

- What more arguments needed?
Outlook

- Efficient evaluation of NIAS requires novel approaches.
  - Innovation concept for safety evaluation is pragmatic and feasible.
  - Increase analytical screening from 10 ppb to 90 µg daily exposure

- Challenges
  - Information transfer through value chain
  - Analysis
  - Relevant exposure data (FACET?)
  - Bioaccumulation, combination toxicity
  - Applying to mixtures of unknown chemical structures
  - Sensitivity genotoxicity assays
  - EFSA

- More demonstrators needed!
- ILSI guidance on NIAS in preparation.
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See you in autumn 2013 (?) at ILSI!

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TTC decision tree; what’s in it?

Exclude:
- high potent genotoxic compounds
  - aflatoxin-like chemicals
  - azoxy compounds
  - N-nitroso compounds

TTC: 0.15 microgram/p/d

Exclude:
- high potent genotoxic compounds
  - aflatoxin-like chemicals
  - azoxy compounds
  - N-nitroso compounds

TTC: 18 microgram/p/d

Exclude:
- high potent genotoxic compounds
  - aflatoxin-like chemicals
  - azoxy compounds
  - N-nitroso compounds

TTC: 90 microgram/p/d

If we can exclude all classes with a threshold, then acceptable exposure to unknowns may go from 0.15 to 90 µg/day! Increase by factor 600!
**LC-ELSD**

**Normal gradient**

**Reversed gradient**