

Extractables and leachables in pharma – **A serious issue**

Leachables are trace amounts of chemicals originating from containers, medical devices or process equipment that end up as contaminants in medicinal products resulting in exposure to patients. We will explain why this is of so much concern and how testing for leachables as well as extractables should be conducted.

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The challenges of leachables have been well known in the food industry for a long time but despite strict regulations, problematic cases gain public attention. Potential exposure to bisphenol A, for example, led the Canadian authorities to ban polycarbonate infant bottles. The EFSA discovered that Germans were exposed to 4-methylbenzophenone in their chocolate muesli. The source was printing ink on the outside of the cardboard box. Recently, Scott Mabury (University of Toronto) found that popcorn bags leach polyfluoroalkyl phosphoric acids, which are absorbed by humans, accumulate in the body and can be metabolized to perfluorinated carboxylic acids which are potentially carcinogenic and hormone disrupting.



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DEFINITION OF EXTRACTABLES AND LEACHABLES

- Extractables are chemical entities, both organic and inorganic, that will extract from components of a container closure system or device into solvents under controlled conditions. They are used to identify and quantify potential leachables.
- Leachables are chemical entities, both organic and inorganic, that migrate from components of a container closure system or device into a drug product over the course of its shelf-life.
- Leachables are usually present in drug product matrices as complex mixtures at trace levels relative to the active pharmaceutical ingredient (API).

In the case of plastic containers, typical extractables and leachables are additives and processing aids such as antioxidants and other stabilizers, plasticizers, emulsifiers, colorants but also monomers and oligomers of the plastic polymer and all kinds of reaction products. Useful information can often be obtained from vendors regarding the formulation of the polymer packaging, but the issue remains complex as the full manufacturing chain from raw materials all the way to a plastic container involves different specialized manufacturers at many steps and full traceability is hard to obtain. Quite generally, containers meant to protect a drug from environmental contamination are actually themselves a source of contamination.

In pharma, not only containers are a source of leachables. Combination products such as inhalers and pens or even more sophisticated medical device equipment like insulin pumps and implants may all leach unwanted chemicals. Also with the advent of disposable equipment, mainly in the manufacturing of biopharmaceuticals, another source of leachables has entered the arena.

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At what stage in the development of a drug E/L becomes an issue, depends on the development program. Certainly, when the final container closure system and/or device is to be selected, E/L programs become mandatory.

SMALL MOLECULE DRUGS AND BIOLOGICS TRIGGER DIFFERENT CONCERNS

Unlike small molecules, biologics can be immunogenic. Leachables might interact with a protein in such a way as to trigger an immune response as observed in the case of Eprex¹. In rare cases patients generated an immune response to an essential natural human protein EPO. The effect had nothing to do with the toxicological properties of the leachables. The consequence was a 100 or so patients requiring blood transfusions for the rest of their lives.

Another recent example is the reaction of the rubber processing chemical thiuram disulfide via disulphide exchange with captopril, a thiol containing angiotensin-converting enzyme inhibitor². Rubber is an exceptionally rich source of diverse classes of processing additives and in both of the above examples rubber was the source of leachables.

Degree of concern associated with the route of administration	Likelihood of packaging component-dosage form interaction		
	HIGH	MEDIUM	LOW
HIGHEST	Inhalation Aerosols and Solutions; Injections and Injectable Suspensions	Sterile Powders and Powders for Injections, Inhalation Powders	
HIGH	Ophthalmic Solutions and Suspensions, Transdermal Ointments and Patches, Nasal Aerosols and Sprays		
LOW	Topical Solutions and Suspensions, Topical and Lingual Aerosols, Oral Solutions, and Suspensions	Topical Powders, Oral Powders	Oral Tablets and Oral (hard and soft Gelatin) Capsules

^ Figure 1: Risk Assessment Table for various dosage forms (adopted from ref. 19)

LEACHING IS NOT LIMITED TO PRIMARY PACKAGING OR TO LIQUIDS

Leaching is facilitated by the exposure of surfaces to liquid. This is well reflected by the generally accepted risk matrix in [Figure 1](#). However mass transfer via the solid state is well known. Similar to the chocolate muesli case, benzophenone and other photoinitiators 1-benzoylcyclohexanol and 2-hydroxy-2-methylpropiophenone from inks used on the labels of HDPE bottles were found to migrate into a solid product³ making regulatory agencies sensitized to this possibility.

OFFICIAL GUIDELINES AND INTEREST GROUPS

Both the USP and the EP contain chapters that deal with the testing of plastic materials, including extraction tests. However only very limited guidelines on E/L for final dosage forms are available from regulators. Probably the most relevant documents are the EMA guideline on plastic immediate packaging materials⁴ which provides a decision tree for when in the EU E/L studies are required and the FDA guideline on container closure systems⁵. Additionally there are guidelines on genotoxic and carcinogenic impurities in drug products in general^{6,7}. None of these guidelines provide any details on how to perform E/L studies.

To fill the gap, various industrial/regulatory groups offer consensus views that help construct convincing arguments that the drug product is safe. Probably the leading group of opinion formers is the influential PQRI working group on leachables and extractables. This group published a recommendation for E/L for orally inhaled nasal drug products (OINDP)^{8,9}. Only recently, PQRI also came up with tentative recom-

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recommendations of parenteral and ophthalmic drug products (PODP)¹⁰. The BioProcess Systems Alliance (BPSA) covers the aspects of E/L of disposable bioreactors¹¹. Last but not least the ELSIE group should be mentioned which is generating databases of extractables and toxicological profiles for the group members¹².

ASSESSMENT OF EXTRACTABLES FORMS THE BASIS OF A SUCCESSFUL LEACHABLES STUDY

Extractable studies are conducted to understand what substances are contained in a material that have the potential to leach into the drug product. The start is a “worst-case scenario” as shown by a “Controlled Extraction Study” under conditions that depend upon the context. The chemical composition of surfaces that come in contact with the drug product, the solubilizing power of the drug product, the duration and conditions of contact are all relevant considerations. This testing is performed under exaggerated conditions of time and temperature in the laboratory using common, neat solvents that bracket the solvating power of the drug. Then, the therapeutic indication dosage form, route of entry into the body and frequency as well as the life

COMPONENTS OF AN EXTRACTABLES STUDY

- 1) Extraction
 - suitable solvents – optimized for the polymer/material
 - vigorous extraction technique(s)
 - to asymptotic levels
- 2) Multiple analytical techniques, GC, LC with different detectors
- 3) Identify principally by MS
- 4) Quantify extractables greater than or equal to the AET (based on the SCT)

expectancy of the patient help establish an acceptable level of risk to be argued on a scientific basis. Reliable identification and sensitive analytical techniques are essential. Expert experimental design avoids unnecessary work and the generation of artifacts through chemical reactions that may trigger false concerns.

TECHNIQUES, DATA GENERATION AND INTERPRETATION

The first major analytical challenge is to identify the substances in the extracts. Efficient identification is critically dependent upon having automated work-flows. The major analytical tools are GC and LC coupled to MS (MS/MS).

Headspace GC-MS	highly volatiles
GC-MS	semi-volatiles
LC-MS	non- volatiles
IC	anions and cations (inorganic or organic)
ICP-MS	elements including heavy metals

Various modes of separation are required owing to the diversity of physical chemical properties encountered. For identification purposes, mass spectrometry is clearly the main work-horse. High mass accuracy is often helpful to narrow down the number of possible chemical structures. An accurate mass combined with a database of typical plastic additives (e.g. NIST for GC-MS, proprietary databases) provides a powerful basis for assignment of chemical structures to chromatographic peaks. The quality of any proprietary database is also a decisive factor in the success of the identification exercise. Following an identification based upon MS, a comparison against an authentic reference standard might be required in order to finally confirm an identity.

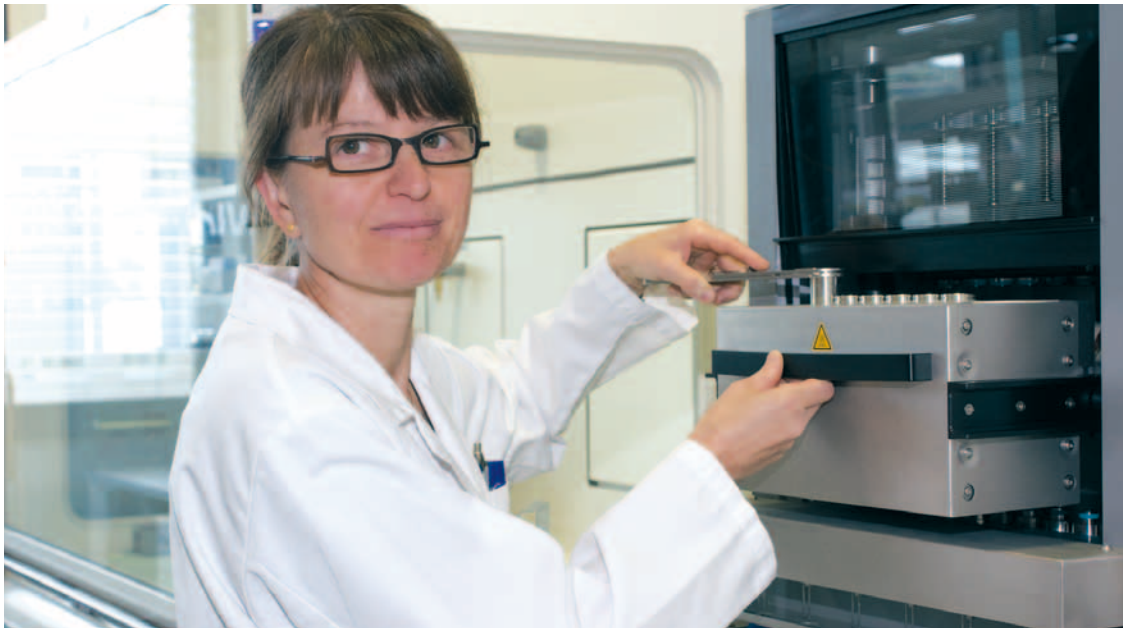
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Accurate quantitation requires comparison against a reference standard but in the absence of such a material relative or semi-quantitation is often an acceptable means to estimate the levels of specific extractables.

TOXICOLOGY AND RISK ASSESSMENT

In order to assess the risk to human health of a substance found in an extractable study, the toxicology of this “potential leachable” must be considered. A toxicological database of extractables is being compiled by ELSIE starting with 15 priority compounds: antioxidants, anti-slip agents, plasticizers, cross-linking agents, lubricants, monomer, starting material, surfactants, and acid scavengers (calcium stearate).

Toxicological information is not often readily available for a number of substances. However, for additives that are considered by the US Environmental Protection Agency (EPA) to be high production volume compounds, there is a lot of public toxicological information available. Compounds that are regulated for food contact applications



^ Accelerated solvent extraction

also often have publically available tox data. The PQRI best practices have established some toxicological thresholds for extractables and leachables for orally inhaled and nasal drug products (OINDP)^{13, 14}:

- 1) The Qualification Threshold (QT) is the level (5 µg/day) below which a given leachable is not considered for safety qualifications unless it presents a structure-activity (SAR) concern.
- 2) The Safety Concern Threshold (SCT) is the dose (0.15 µg/day) below which a leachable would present negligible concern for adverse carcinogenic and noncarcinogenic effects.
- 3) Known highly toxic substances such as PAHs, nitrosamines and 2-mercaptobenzothiazole) are considered to be “special case compounds” for orally inhaled and nasal drug applications and should be considered on a case-by-case basis.
- 4) By using one of these toxicological thresholds as well as dosing information, the Analytical Evaluation Threshold (AET) can be calculated. The AET is the level at or above which an OINDP pharmaceutical development team should identify and quantify a particular extractable and/or leachable and report it for potential toxicological assessment.

Above limits apply irrespective of the duration of the exposure. A short-term exposure (e.g. vaccination) would however be clearly less risky than a life-long exposure (e.g. a drug against hypertension). For drugs that are life-saving in an acute situation or in cases where life expectancy is limited much higher limits have in the past been accepted. Risk assessment is a multi-variate task and sound scientific reasoning must be used to demonstrate that any risks are acceptable.

LEACHABLES

The leachable study is conducted in order to monitor any substances that leach out of a material into the final drug product in its commercial packaging (primary and secondary) and are thus exposed to patients. The smart way to conduct a regulatory leachables study is as part of a regulatory stability study. The substances of concern and to be controlled will have been identified via toxicological consideration of the extractables data. A simple example of how analytical evaluation threshold is set follows:

If we take the SCT proposed by PQRI of 0.15 µg per day for an individual leachable in an OINDP, an MDI with 0.5 mL of drug product in a canister that has 200 actuations

COMMON ABBREVIATIONS USED IN EXTRACTABLES AND LEACHABLES

	ABBREVIATION	NOTES
Analytical Evaluation Threshold	AET	Sensitivity of analytical method for leachables studies (see PQRI)
Drug Product	DP	Formulated active ingredient
Orally Inhaled and Nasal Drug Product	OINDP	Common dosage forms
Metered Dose Inhaler	MDI	Device delivering a specific amount of medication to the lungs
Safety Concern Threshold	SCT	Acceptable daily intake for a carcinogen (see PQRI)
Total Daily Intake	TDI	Mass ingested per day per person
Threshold of Toxicological Concern	TTC	Same as TDI

with a recommended daily dose of 10 actuations would have an estimated AET of 6 µg/mL and a final AET of 3 µg/mL.

The impact of drug formulation upon leachables was shown in a study¹⁵ using rubber exposed to 11 common formulations. Key findings were drug product additives that alter the polarity of the formulation could impact the leachables profile, for example; Tween 80 significantly increased the leachables 2-methylpentane, 3-methylpentane, hexane, methylcyclopentane, and cyclohexane but had no impact on BHT. The bulking agents sucrose, mannitol, and trehalose also led to different levels of leachables but trehalose gave the minimum levels.

In the following sections a few special topics are mentioned that deserve special attention.

PREFILLED SYRINGES

These devices are increasingly popular. Potential contaminants are inorganic oxides and ions from borosilicate glass, organics from the rubber plunger, Fe, Cr, Mn, Ni and Mo from the stainless steel needle; adhesives (organic oligomers and polymers such as acrylates that are processed via curing process); silicone oil (polydimethyl siloxane) to lubricate the barrel, syringe tools including tungsten pin and nylon pins. Metals and ions such as Fe and Mn may catalyze oxidation of proteins. Where polymers are used rather than glass the usual scenario of investigation organic molecule E/L will apply. Interactions with ions or other molecules might lead to aggregation in the case of biologics.

CONTROL OR QUALIFICATION OF MATERIALS

The Polymerforum Group engages the polymer supplier chain in understanding of the needs of pharma. A minor change in a polymer manufacturing chain might trigger product recalls and lengthy investigations. Control of materials via extractables testing should use validated analytical methods (in accordance to ICH guidelines) that are also capable of detecting new substances.

Many materials are used such as glass, natural or synthetic rubbers, polyethylene, polyethylene terephthalate, and polypropylene (see US Pharmacopeia <661 >), polyvinylchloride, polymethylacrylates, polyolefins, co-polymers such as acrylonitrile-butadienestyrene. These materials are themselves complex formulations containing numerous impurities and additives that serve a variety of purposes therefore representing thousands of possible chemical structures. Regulators also have compliance requirements for container closure systems that are also medical devices. Various sections of ISO10993 apply to such combination products. Companies must provide quantitative data on the chemical composition of the materials going into their devices. In all cases, E/L are not limited to raw materials but also include any compositional changes that are likely to occur in use.

ORGANIZATIONS INVOLVED WITH EXTRACTABLES AND LEACHABLES

	ABBREVIATION	NOTES
American Association for Pharmaceutical Science	AAPS	International forum – exchange of knowledge among scientists – discovery, development and manufacture of pharmaceutical products www.aaps.org
Bio-Process Systems Alliance	BPSA	Inter-company group supporting the use of disposable reactor technology www.bpsalliance.org
European Food Safety Authority	EFSA	An EU official body www.efsa.europa.eu
Extractables and Leachables Safety Information Exchange	ELSIE	Inter-company group producing databases of 1) extractables from selected plastics 2) toxicology for selected substances www.elsiedata.org
International Pharmaceutical Aerosol Consortium on Regulation and Science	IPAC-RS	International association of innovator and generic companies that develop, manufacture or market orally inhaled and nasal drug products www.ipacrs.com www.ipacrs.com/PDFs/Device%20Paper.pdf
AAPS Inhalation Technology Focus Group	ITFG	www.aaps.org/inside/focus_groups/IT/index.asp
Polymerforum		Annual meetings and continuous dialogue in between polymer suppliers and the pharma industry www.ipacrs.com/PDFs/Materials/8-Polymer%20Forum%20-%20Tjader.pdf
National Institute of Standards and Technology	NIST	US agency responsible for advancing measurement science, standards, and technology www.nist.gov
Product Quality Research Institute	PQRI	Collaboration between FDA's Center for Drug Evaluation and Research, Industry, and Academia www.pqri.org

DISPOSABLE BIOREACTORS

Are we only concerned with the final container/delivery system? Unfortunately not! With the increasing popularity of disposable bioreactors for the production of biologics the various plastics – bags, tubing, filters are sources of potential contamination. It may be possible to adopt a process validation approach to show that contaminants are removed by down-stream processing. Interestingly, the BPSA^{11, 16} advocates the use of a less extreme range of extraction conditions than recommended for drug products.

An essential principle is “fit for purpose” solutions meeting regulatory aims without “over-engineering.”

THE SOLVIAS APPROACH

Solvias has worked on E/L over the years and offers expert service incorporating smart practices for sample preparation such as accelerated solvent extraction and a full range of analytical technologies. Beyond the NIST database, Solvias has a large in-house proprietary mass spectral database with over 2000 entries for using standardized LCMS and GCMS methods for the automated reliable identification of polymer additives and their degradation products.

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EXPERTISE AND TECHNOLOGY ARE THE ONLY SHORTCUTS

Serious contamination of food and medicine may rapidly become global knowledge via today’s information superhighways. The challenges should not be underestimated and must be addressed scientifically and methodically via the decision-trees such as are described by PQRI.

The key overarching E/L concepts are risk assessments and control systems. AETs are based upon SCTs. Studies are performed in a tiered multidisciplinary approach that aims to satisfy the regulators that the risks have been identified and controlled.

Resources can be minimized and risks managed through adopting state-of-the-art methodologies within fully engineered work-flows designed by experts and supported by automation. Thereafter effective advocacy and consultation with the regulators maximizes the probability of winning market authorization.

Taking into account all of the above considerations, it can be challenging to effectively design a program that will adequately address and evaluate the E/L issues associated with the container closure system, delivery devices, and processing equipment. It is very important to partner with a CRO/CTO that has the knowledge and experience to bring your product to market in an efficient manner. •

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