8 SEPTEMBER 2006

SAFETY THRESHOLDS AND BEST PRACTICES FOR EXTRACTABLES AND LEACHABLES IN ORALLY INHALED AND NASAL DRUG PRODUCTS

Submitted to the PQRI Drug Product Technical Committee,
PQRI Steering Committee, and U.S. Food and Drug Administration
by the
PQRI Leachables and Extractables Working Group

Daniel Norwood (IPAC-RS), Chair
Douglas Ball (IPAC-RS)
James Blanchard (IPAC-RS)
Lidiette Celado (AAPS)
T.J. Deng (Lab)
Fran DeGrazio (PDA)
Bill Doub (FDA)
Thomas Feinberg (AAPS)
Alan Hendricker (Lab)
Jeff Hrkach (AAPS)
Roger McClellan (University of New Mexico)
Timothy McGovern (FDA)
Diane Paskiet (PDA)
David Porter (USP)
Michael Ruberto (Lab)
Alan Schroeder (FDA)
Mark Vogel (PhRMA)
Qingxi Wang (PhRMA)
Ronald Wolff (IPAC-RS)
Melinda Munos (IPAC-RS)
Lee Nagao (IPAC-RS)

The views expressed in this document are not necessarily those of the US Food and Drug Adminstration.

TABLE OF CONTENTS

Part 3: Best Practices for Extractables and Leachables Studies for Orally Inhaled and Nasal Drug Products

Part 4: Appendices

FORWARD

Leachables and Extractables (L&E) issues represent some of the most significant challenges facing a pharmaceutical development team responsible for the registration and manufacture of Orally Inhaled or Nasal Drug Products (OINDP). In contrast to drug substance or excipient related impurities, organic leachables and extractables represent a diversity of chemical structures and compound classes, and are potentially present at widely varying concentrations in any particular OINDP. To further complicate the picture, regulatory concern regarding leachables and extractables in OINDP is directly related to the particular type of OINDP, e.g., Metered Dose Inhaler, Dry Powder Inhaler, Inhalation Solution, Nasal Spray. Guidance documents, both fully released and in draft form, from the United States Food and Drug Administration (USFDA) have significantly clarified the pharmaceutical development process for OINDP, including leachables and extractables issues. However, significant uncertainties remain. These uncertainties can delay pharmaceutical development programs and complicate the regulatory review and approval process.

The Product Quality Research Institute (PQRI) Leachables and Extractables Working Group was established with the intent of reducing as much as possible the remaining uncertainty in the OINDP pharmaceutical development process for leachables and extractables, using science based and data driven approaches. The Working Group is made up of highly experienced scientists including toxicologists, analytical chemists, and others, from industry, government, and academia. This recommendation document to the USFDA represents the culmination of the Working Group's efforts. The document includes recommended exposure thresholds above which individual organic leachables in an OINDP must be qualified and/or evaluated for safety concern. A systematic process for leachables safety assessment is also presented. These "safety thresholds" are linked to a recommended Analytical Evaluation Threshold (AET) which for the first time provides guidance on the perplexing question of: How low do you go?

In addition to these threshold recommendations, the document proposes "best practices" in areas such as: OINDP Component Selection, Controlled Extraction Studies, Leachables Studies, and Routine Quality Control Methods. The best practices recommendations are based on a great deal of laboratory work, including comprehensive Controlled Extraction Studies and simulated leachables studies, performed by volunteer laboratories. Selected data from these studies are included in this document to illustrate and discuss the key recommendations and observations.

The recommendations presented in this document are not intended to be prescriptive. The Working Group recognizes that there can be product specific approaches to extractables and leachables risk assessment and testing, and that these can and should be discussed between the sponsor and appropriate regulatory authority.

The members of the Working Group wish to acknowledge the Product Quality Research Institute and its member organizations for providing the forum and mechanisms which make a collaboration such as this possible. We also wish to acknowledge the dedicated scientists in the volunteer laboratories and the science advisors from the International Pharmaceutical Aerosol Consortium on Regulation and Science (IPAC-RS) Secretariat, all of whom contributed enormously to this effort. The Working Group hopes that the recommendations contained in this

document will serve to remove uncertainty from the pharmaceutical development process for OINDP, thereby facilitating the approval and manufacture of safe, effective, and quality inhalation drug products.

On behalf of the PQRI Leachables and Extractables Working Group

Daniel L. Norwood, Ph.D.

Chair, PQRI Leachables and Extractables Working Group
Representing the International Pharmaceutical Aerosol Consortium for Regulation and Science

ACKNOWLEDGEMENTS

The development of these recommendations could not have been achieved without the hard work and dedication of scientists from FDA, industry and academia, who volunteered their time to conduct scientific studies, generate data, and review the documents. These activities were fundamental and critical to the development of consensus within the Working Group, and to development of the final recommendations.

The individuals who supervised and performed the extractables and simulated leachables studies are:

Boehringer Ingelheim
Dr. Alice Granger
Mr. Keith McKellop
Dr. Fenghe Qiu
Mr. James Mullis

Merck
Dr. Tiebang Wang
Mr. Decheng Ma
Dr. Anne Payne

Cardinal Health
Dr. Alan Hendricker
Ms. Andrea Deal
Dr. Zhen Mei
Dr. Rob Piccoli
Ms. Amanda Ryder

West Monarch Analytical Laboratories
Ms. Lisa Bavis
Mr. Ron Plenzler
Ms. Laura Stubbs

Ciba Expert Services
Dr. John Hand
Mr. David Olenski
Dr. Michael Ruberto

PPD
Dr. Tian-Jing Deng
Dr. Sue Chudasama
Dr. Shuang Li

The individuals who performed the structure activity relationship studies are:

FDA
Dr. R. Daniel Benz
Dr. Joseph Contrera
Dr. Naomi Kruhlak
Dr. Edwin Matthews

Pfizer
Dr. Nigel Greene

Test articles were generously provided by Owens Illinois; Valois, Inc; and West Pharmaceuticals. Ms. Jennifer Hicks (Chevron-Phillips) provided the polypropylene resin and Mr. Jim Mierzwiak (Owens Illinois) molded the plaques.

Several experts generously provided their time and expertise to critically review drafts of the document. Dr. David Alexander (DA Non-clinical Safety, Ltd), Dr. Elmer Mirro (Schering Plough), Dr. Mark Utell (University of Rochester), Dr. David Gaylor (Sciences International), Dr. James MacDonald (Schering Plough), and Dr. Raymond Stoll (Stoll and Associates, LLC) reviewed drafts of the threshold justification. Dr. Dennis Jenke (Baxter), Mr. Jason Creasey

(Glaxo Smith Kline), Ms. Gaby Reckzuegel (Boehringer Ingelheim), Dr. Holger-Thorsten Steinfuehrer (Boehringer Ingelheim), Mr. Thomas Egert (Boehringer Ingelheim), and Mr. James O. Mullis (Boehringer Ingelheim) reviewed drafts of the best practices recommendations.

Dr. Lee M. Nagao, Ms. Melinda Munos, and Dr. Svetlana Lyapustina of the IPAC-RS Secretariat drafted, edited, and critically reviewed the document; coordinated and managed all activities of the Working Group, and drove the process forward.

The Working Group thanks all of these scientists for their contributions of expertise, energy and commitment to this important effort.

PART 1

INTRODUCTION AND SUMMARY OF RECOMMENDATIONS

I. INTRODUCTION

This document presents the recommendations of the Product Quality Research Institute (PQRI) Leachables and Extractables Working Group, addressing the development of scientifically supported analytical testing and safety evaluation thresholds for leachables and extractables in Orally Inhaled and Nasal Drug Products (OINDP). Also presented, are recommendations for industry "best practices" in all OINDP pharmaceutical development areas related to extractables and leachables. The threshold and best practices recommendations are based on the working Group's evaluation of the current state of scientific knowledge, original laboratory data developed by the Working Group, and the regulatory approval and product development experiences of individual Working Group members.

The PQRI Leachables and Extractables Working Group consists of scientists from FDA, industry and academia, all of whom have experience in various aspects of leachables and extractables work in pharmaceutical development. PQRI established the Leachables and Extractables Working Group in 2001 to develop the aforementioned thresholds for leachables and extractables, and to propose recommendations for leachables and extractables testing that would clarify and provide a rationale for existing FDA guidance on this subject. Existing guidance is contained in the *Draft Guidance for Industry Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products - Chemistry, Manufacturing, and Controls Documentation, and the Guidance for Industry Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products - Chemistry, Manufacturing, and Controls Documentation.*[1,2] Establishment of scientifically based analytical and safety evaluation thresholds and best practice recommendations for leachables and extractables testing will serve to reduce uncertainty in the regulatory application and review process, an effort in support of current Agency initiatives.[3,4]

Note that best practice recommendations for leachables and extractables testing included in this document (such as for Controlled Extraction Studies, Leachables Studies, and Routine Extractables Testing for components) are not meant to be prescriptive or to exclude other scientifically valid approaches, analytical techniques/methods, or control strategies. These recommendations represent a consensus within the Working Group on current best practices within the pharmaceutical industry and are designed to reduce the level of uncertainty within the OINDP development process. Note also that this document presents science and experience based recommendations for best practices and thresholds and is not an FDA regulatory policy document.

A. Scope

The scope of this document includes all Orally Inhaled and Nasal Drug Products (OINDPs) currently in use or under development and their various container/closure and delivery systems. These include Metered Dose Inhalers (MDIs), Dry Powder Inhalers (DPIs), Inhalation Solution, Suspension, and Spray products and Nasal Sprays. The recommendations are applicable to components of the OINDP container/closure system (the "components") that are in contact with the formulation, the patient's mouth or the nasal mucosa, or that are deemed "critical" to the functionality of the drug product. Ancillary components required by the OINDP

label, including specifically named nebulizers and spacers, are covered by these recommendations. The analytical testing and safety evaluation thresholds, and best practice recommendations, presented in this document were developed using laboratory data and other scientific information specifically relevant to OINDP. Therefore, these thresholds and best practices apply only to OINDP and not to any other drug product types, e.g., injectables, solid oral dosage forms.

Furthermore, the thresholds proposed in this document are applicable only to organic leachables and extractables from OINDP. The thresholds are not applicable to identification and qualification of solvents, and drug substance or drug product impurities and degradents, which are covered in the ICH Q3 guidelines. Further, the Working Group recognizes that dissolved metals and foreign particulate matter are also important matters for OINDP pharmaceutical development. This recommendation document, however, focuses only on organic leachables and extractables. Based on the collective experiences of the Working Group members, including FDA members, organic leachables were considered to be the main challenge for OINDP pharmaceutical development teams and the Working Group therefore determined to focus its efforts there. However, the basic approach to dissolved metals (other than techniques) should be similar. It was further agreed by the Working Group that "foreign particulate matter" (including metallic particles) are not within the remit of this working group.

B. Hypothesis

The Working Group first developed and proposed the following two-part hypothesis for scientific evaluation:[5]

1. *Scientifically justifiable thresholds based on the best available data and industry practices can be developed for:*

   (a) *the reporting and safety qualification of leachables in orally inhaled and nasal drug products, and*

   (b) *the reporting of extractables from the critical components used in corresponding container/closure systems.*

   Reporting thresholds for leachables and extractables should include associated identification and quantitation thresholds.

2. *Safety qualification of extractables would be scientifically justified on a case-by-case basis.*

The practical rationale for development of these analytical testing and safety evaluation thresholds is that analytical techniques are increasingly sophisticated and capable of detecting and identifying individual chemical entities at extremely low levels, e.g., sub-picogram. However, it is generally accepted that there are levels of chemicals below which the risks to human health are so negligible as to be of no consequence. The Working Group proposes that leachables present in OINDP, when held below data-supported threshold levels, are generally not of concern.

Note that certain compound classes of potential extractables and leachables with special safety concerns, e.g., N-nitrosamines, Polynuclear Aromatic Hydrocarbons (PAHs or PNAs), 2-mercaptobenzothiazole, may require lower thresholds than those proposed in this document, along with dedicated methods, appropriate specifications, appropriate qualifications, and risk assessments.

C. Investigation of Hypothesis

To investigate the hypothesis, the Working Group performed analytical laboratory experiments and toxicology/safety database reviews. The Working Group toxicologists collected and assessed data from well-established databases of safe exposure levels and applied conservative risk analysis procedures to these data. Through this process, they developed safety evaluation and qualification thresholds.

The Working Group chemists conducted protocol-based Controlled Extraction Studies and simulated Leachables Studies. They optimized and validated the methods for the quantitative Controlled Extraction Studies and collected and assessed the data generated from both the extraction and leachables studies. The simulated leachables studies were conducted under conditions appropriate for an MDI drug product because MDIs provide the worst-case conditions for observing a qualitative correlation between leachables and extractables. That is, unlike DPIs and other OINDP delivery systems, there is generally a one to one qualitative correlation between extractables and leachables in any given MDI drug product.

From the thresholds developed by the toxicologists and the data from the Controlled Extraction and simulated Leachables Studies, the chemists developed a process for determining analytical thresholds for extractables and leachables and recommendations on best practices for conducting extractables and leachables studies. These best practice recommendations provide guidance for all OINDP on how to conduct Controlled Extraction Studies and Leachables Studies, establish correlations between extractables and leachables profiles, and establish and use the analytical thresholds.

II. BACKGROUND

A. Extractables and Leachables

Extractables are compounds that can be extracted from OINDP device components or surfaces of the OINDP container/closure system in the presence of an appropriate solvent(s) and/or condition(s). Thus, extractables are individual chemical entities that can be extracted from individual component types, e.g., rubber seals, plastic valve parts, of an OINDP container/closure system under relatively vigorous laboratory conditions using appropriate solvents or solvent systems. Extractables can, therefore, be considered as potential leachables in OINDP.

Leachables in OINDP are compounds which are present in the drug product due to leaching from container/closure system components. Leaching can be promoted by the formulation, or components of the formulation, e.g., CFC or HFA propellants in MDIs. Leachables are often a subset of, or are derived directly or indirectly from extractables. Due to the time-dependent nature of the leaching process, leachables appear in an OINDP formulation

over the shelf-life of the product as determined during appropriate stability and accelerated stability studies.

As some extractables and leachables may affect product quality, safety and efficacy, regulatory guidances have provided recommendations regarding their analysis and toxicological safety assessment, i.e., qualification.

B. Extraction Studies and Leachables Studies

Extraction studies (often called controlled or control extraction studies -- in this document they are referred to as "Controlled Extraction Studies") are intended to provide a thorough understanding of potential leachables from appropriate OINDP container/closure system components early in the pharmaceutical development process. In these studies, components must be placed in a variety of solvents with a range of polarities and then subjected to vigorous laboratory extraction conditions in order to maximize the levels of extractables and provide a "worst-case" picture of potential leachables levels. The component extracts are analyzed to identify and quantify individual extractables.

An analytical threshold for extractables would be a useful benchmark at this point, to guide the sponsor of the pharmaceutical development program in choosing which extractables to identify, quantify, and assess for safety/toxicology concerns.

Leachables studies are often not conducted until later in the pharmaceutical development program. In these studies, drug product is stored on stability under a variety of controlled environmental conditions and analyzed for leachables (both qualitatively and quantitatively) at multiple time-points over the anticipated shelf-life of the drug product. At this point, safety evaluation and qualification, and analytical thresholds would be particularly useful to the sponsor.

C. Potential Sources of Extractables and Leachables

Potential sources of extractables and leachables in various OINDP are presented in Table 1. This list is not exhaustive, and other sources of extractables and leachables are possible for each dosage form.

Table 1. Potential Sources of Extractables and Leachables from OINDP

Dosage Form	Potential Source of Extractables and/or Leachables
MDIs	· Metal components, e.g., MDI valve components, canisters - Residual cleaning agents, organic surface residues, e.g., heavy oils or surface treatments of any type that are in contact with the formulation or the patient - coatings on internal canister surface · Elastomeric container/closure system components, e.g., gaskets, seals, etc. - Chemical additives, including antioxidants, stabilizers, plasticizers, etc. - Trace level contaminants and reaction products contained within

	chemical additives - Monomers and oligomers from the elastomer - Secondary reaction products from the curing process · Plastic/polymeric container/closure system components, e.g., plastic MDI valve components, mouthpieces, plastic container material - Chemical additives, including antioxidants, stabilizers, plasticizers, etc. - Trace level contaminants and reaction products contained within chemical additives - Monomers and oligomers from the polymeric material - Pigments · Processing aids, e.g., chemicals applied to surfaces of processing/fabrication machinery, or directly to components - Mould release agents - Lubricants
DPIs	· Elastomeric container/closure system components, e.g., gaskets, seals - Chemical additives, including antioxidants, stabilizers, plasticizers, etc. - Trace level contaminants and reaction products contained within chemical additives - Monomers and oligomers from the elastomer - Secondary reaction products from the curing process · Plastic/polymeric container/closure system components, e.g., plastic components, including mouthpieces and plastic container material - Chemical additives, including antioxidants, stabilizers, plasticizers, etc. - Trace level contaminants and reaction products contained within chemical additives - Monomers and oligomers from the polymeric material - Pigments · Processing aids, e.g., chemicals applied to surfaces of processing/fabrication machinery, or directly to components - Mould release agents - Lubricants · Blisters or capsules containing individual doses of drug product - Chemical additives - Adhesives and glues
Inhalation solutions, suspensions and sprays	· Plastic/polymeric container/closure system components, e.g., plastic components, including mouthpieces and plastic container material - Chemical additives, including antioxidants, stabilizers, plasticizers, etc. - Trace level contaminants and reaction products contained within chemical additives - Monomers and oligomers from the polymeric material - Pigments · Labels, e.g., paper labels on inhalation solution plastic containers - Inks - Adhesives/glues · Processing aids, e.g., chemicals applied to surfaces of processing/fabrication machinery, or directly to components

	- Mould release agents - Lubricants
Nasal sprays	· Plastic/polymeric container/closure system components, e.g., plastic components, including spray nozzles and plastic container material - Chemical additives, including antioxidants, stabilizers, plasticizers, etc. - Trace level contaminants and reaction products contained within chemical additives - Monomers and oligomers from the polymeric material - Pigments · Elastomeric container/closure system components, e.g., gaskets, seals - Chemical additives, including antioxidants, stabilizers, plasticizers, etc. - Trace level contaminants and reaction products contained within chemical additives - Monomers and oligomers from the elastomer - Secondary reaction products from the curing process · Labels, e.g., paper labels on nasal spray plastic containers - Inks - Adhesives/glues · Processing aids, e.g., chemicals applied to surfaces of processing/fabrication machinery, or directly to components - Mould release agents - Lubricants

III. CONCLUSIONS AND RECOMMENDATIONS

Through investigation of the hypothesis, the Working Group formulated several conclusions and proposals addressing safety thresholds, safety qualification, and best practices for extractables and leachables testing. The Recommendations are divided into two main parts, which cover these topics: (i) the derivation and justification of safety thresholds, and (ii) best practices for extractables and leachables studies in pharmaceutical development programs for OINDP. The key conclusions and recommendations are listed below.

A. Thresholds

· Scientifically justifiable safety evaluation and qualification thresholds for leachables in OINDP can be established. The Working Group proposes a Safety Concern Threshold (SCT) of 0.15 µg per day, and a Qualification Threshold (QT) of 5 µg per day for an individual leachable in an OINDP.

· The SCT is defined as the threshold below which a leachable would have a dose so low as to present negligible safety concerns from carcinogenic and noncarcinogenic toxic effects.

- The QT is defined as the threshold below which a given leachable is not considered for safety qualification (toxicological assessments) unless the leachable presents structure-activity relationship (SAR) concerns.

- These safety thresholds are represented as absolute exposures, expressed in total daily intake (total exposure per day). They must be converted into relative amounts, expressed in terms such as amount of an individual leachable in a particular drug product, e.g., µg per canister in an MDI, to be useful to analytical chemists conducting leachables and extractables studies. This conversion is performed by using information on the drug product configuration such as the number of actuations per canister, number of doses per day, number of actuations per dose, number of actuations per day, etc. The converted SCT, which should be used by the analytical chemists is called the Analytical Evaluation Threshold (AET).

- Scientifically justifiable analytical thresholds for extractables and leachables in OINDP can be established. These analytical thresholds, however, should not be considered "reporting" or "identification" thresholds as traditionally used in other applications such as in the ICH process for limits on drug substance-related impurities and degradants. To avoid confusion with the ICH terms, the Working Group proposes the AET. The AET is developed during extractables studies and is applied to both extractables and leachables.

- The AET is defined as the threshold at or above which a chemist should begin to identify a particular leachable and/or extractable and report it for potential toxicological assessment.

- The AET will vary depending on (i) the particular drug product configuration and (ii) the method(s) used to detect and quantify the extractables and leachables. The methods used will affect the AET value because of the analytical uncertainty inherent in the response factors of individual leachables (or extractables) analyzed by any given analytical technique/method.

B. Integration of Safety Evaluation

- Safety evaluation or "risk assessment" should be integrated into the pharmaceutical development process so that extractables (and potential leachables) may be assessed for safety at early and appropriate stages of development. This evaluation can be performed at three key points in the pharmaceutical development process:

  - During the selection of components and materials;

  - On extractables during Controlled Extraction Studies; and

  - On leachables during Leachables Studies for drug product registration.

C. Components

- The pharmaceutical development team should obtain all available information on the composition and manufacturing/fabrication processes for each component type to the extent possible, and determine which components are "critical."

- Component formulation should inform component selection.

- Risk Assessment should be performed during the selection of components and materials.

- Extractables testing, including Controlled Extraction Studies and the development and validation of Routine Extractables Testing methods, should be accomplished for all critical OINDP components.

D. Controlled Extraction Studies

- Controlled Extraction Studies should employ vigorous extraction with multiple solvents of varying polarity.

- Controlled Extraction Studies should incorporate multiple extraction techniques.

- Controlled Extraction Studies should include careful sample preparation based on knowledge of analytical techniques to be used.

- Controlled Extraction Studies should employ multiple analytical techniques.

- Controlled Extraction Studies should include a defined and systematic process for identification of individual extractables.

- Controlled Extraction Study "definitive" extraction techniques/methods should be optimized.

- During the Controlled Extraction Study process, sponsors should revisit supplier information describing component formulation.

- Controlled Extraction Studies should be guided by an Analytical Evaluation Threshold (AET) that is based on an accepted safety concern threshold.

- Polycyclic Aromatic Hydrocarbons (PAH's; or Polynuclear Aromatics, PNA's), N-nitrosamines, and 2-mercaptobenzothiozole (MBT) are considered to be "special case" compounds, requiring evaluation by specific analytical techniques and technology defined threshold.

- Qualitative and quantitative extractables profiles should be discussed with and reviewed by pharmaceutical development team toxicologists so that any potential safety concerns regarding individual extractables, i.e., potential leachables, are identified early in the pharmaceutical development process.

E. Leachables Studies and Routine Extractables Testing

- Analytical methods for the qualitative and quantitative evaluation of leachables should be based on analytical technique(s)/method(s) used in the Controlled Extraction Studies.

- Leachables Studies should be guided by an Analytical Evaluation Threshold (AET) that is based on an accepted safety concern threshold.

- A comprehensive correlation between extractables and leachables profiles should be established.

- Specifications and acceptance criteria should be established for leachables profiles in OINDP as required.

- Analytical methods for Routine Extractables Testing should be based on the analytical technique(s)/method(s) used in the Controlled Extraction Studies.

- Routine Extractables Testing should be performed on critical components using appropriate specifications and acceptance criteria.

- Analytical methods for Leachables Studies and Routine Extractables Testing should be fully validated according to accepted parameters and criteria.

- Polycyclic Aromatic Hydrocarbons (PAH's; or Polynuclear Aromatics, PNA's), N-nitrosamines, and 2-mercaptobenzothiazole (MBT) are considered to be "special case" compounds, requiring evaluation by specific analytical techniques and technology defined thresholds for Leachables Studies and Routine Extractables Testing.

- Qualitative and quantitative leachables profiles should be discussed with and reviewed by pharmaceutical development team toxicologists so that any potential safety concerns regarding individual leachables are identified as early as possible in the pharmaceutical development process.

IV. EXAMPLE PHARMACEUTICAL DEVELOPMENT AND QUALIFICATION PROCESS FOR LEACHABLES AND EXTRACTABLES IN OINDP

The safety thresholds, safety qualification process, and best practices recommendations contained in Part II and Part III can be applied in a comprehensive process for conducting extractables and leachables studies and safety qualification of leachables, incorporating the AET, the SCT and the QT. Note that the proposed safety and analytical thresholds cannot meaningfully be used outside of a cohesive and scientifically sound process for conducting extractables and leachables studies. A comprehensive step-wise process is proposed here and depicted schematically in Figures 1 and 2. Note that this process constitutes a proposal by the Working Group and is not meant to be prescriptive:

1. The sponsor should first select the appropriate components, e.g., elastomeric seals, canisters, mouthpiece, plastic containers for inhalation solutions, based on functionality, availability, physicochemical makeup, and other appropriate factors,

and obtain as much information as possible from the component supplier(s) as to the qualitative and quantitative chemical formulation, and manufacturing/fabrication processes of each component type selected. Compositional information from the supplier should be reviewed by toxicologists for risk assessment on individual ingredients during the component/material selection process.

2. The sponsor should understand the drug product configuration, e.g., number of doses per day, total number of doses in a drug product unit.

3. The sponsor should then conduct Controlled Extraction Studies, and consider reporting individual identified extractables for risk assessment.

   (a) During this step, the sponsor should first estimate the AET. Estimating the AET for extractables allows the sponsor to develop a benchmark or threshold which allows preliminary determination of which extractables should be identified and quantified. All extractables greater than or equal to the estimated AET should be identified, to the extent possible. The AET can be estimated from the SCT by converting the SCT from units of daily exposure (µg/day) to units of amount per product unit or dose, e.g., µg/canister, µg/dose, µg/blister. This value is then converted into amount per gram of component, e.g., µg/gram, using the weight and amount of component used per drug product. This resulting value is the estimated AET. The required sensitivity of the analytical method(s) (the LOQ) can then be determined from the estimated AET.

   (b) Qualitative studies should be performed using a variety of solvents and extraction methods, and several complementary analytical techniques/methods. Extractables greater than or equal to the estimated AET should be identified.

   (c) The sponsor should then conduct quantitative Controlled Extraction Studies. Appropriate extraction methods identified in the qualitative Controlled Extraction Studies should be optimized. Optimization consists of selecting the extraction method providing the greatest number and concentration of extractables, and optimizing the extraction conditions to achieve asymptotic levels of extractables. This process allows the sponsor to predict a worst-case leachables profile. The precision and accuracy of the analytical methods based on those used in the qualitative studies, should be verified.

   (d) The uncertainty of each analytical method used for definitive extractables profiling should be estimated. One way to accomplish this is to develop a response factor database of extractables using authentic standards (where available). The estimated uncertainty, for the given method, should be applied to the estimated AET to calculate the final AET. This determination allows the sponsor to refine the original estimated AET, and if necessary, to identify any extractables that were not assessed previously.

   (e) The analytical methods should be used to detect and quantify those compounds greater than or equal to the final AET.

(f) Extractables detected and quantified in the Quantitative Controlled Extraction studies that are greater than or equal to the AET for extractables should be discussed with toxicologists to determine appropriate further action.

(g) It is essential to report extractables for risk assessment at this early stage, as doing so will allow the sponsor to understand and address potential safety concerns early in the pharmaceutical development process.

4. Extraction and analytical methods for Routine Extractables Testing should be established based on methods developed in the Controlled Extraction Studies, and validated according to established parameters. Extractables profiles from these routine studies should be monitored for anomalous results. To aid in determination of anomalies, the sponsor should develop a profile specification, which should include acceptance criteria for known extractables as well as "unspecified" extractables, i.e., extractables not identified in qualitative Controlled Extraction Studies. Additionally, the sponsor should develop a procedure for investigating an obvious change in a component's extractable profile which does not necessarily result in a batch failure (often termed an "out of trend investigation"). Following establishment of a correlation between leachables and extractables and a profile specification, Routine Extractables Testing for quality control should be performed. Based on Controlled Extraction Studies and leachables studies, acceptance criteria for leachables and extractables should be developed.

5. After Controlled Extraction Studies have been completed, the sponsor should conduct Leachables Studies on drug product.

(a) Analytical methods to detect and quantify leachables can be based on the methods developed in Controlled Extraction Studies. These methods should be sensitive and validated according to established parameters, using major extractables as model compounds, i.e., a selection of those identified in the Controlled Extraction Studies equal to or greater than the final AET.

(b) Leachables Studies should be conducted with drug product stored under a variety of controlled conditions as part of formal stability studies. These studies should be performed in accordance with the ICH Q1A(R2) guidance document. Results from Leachables Studies conducted on stability samples should be correlated to extractables profiles generated from Controlled Extraction Studies.

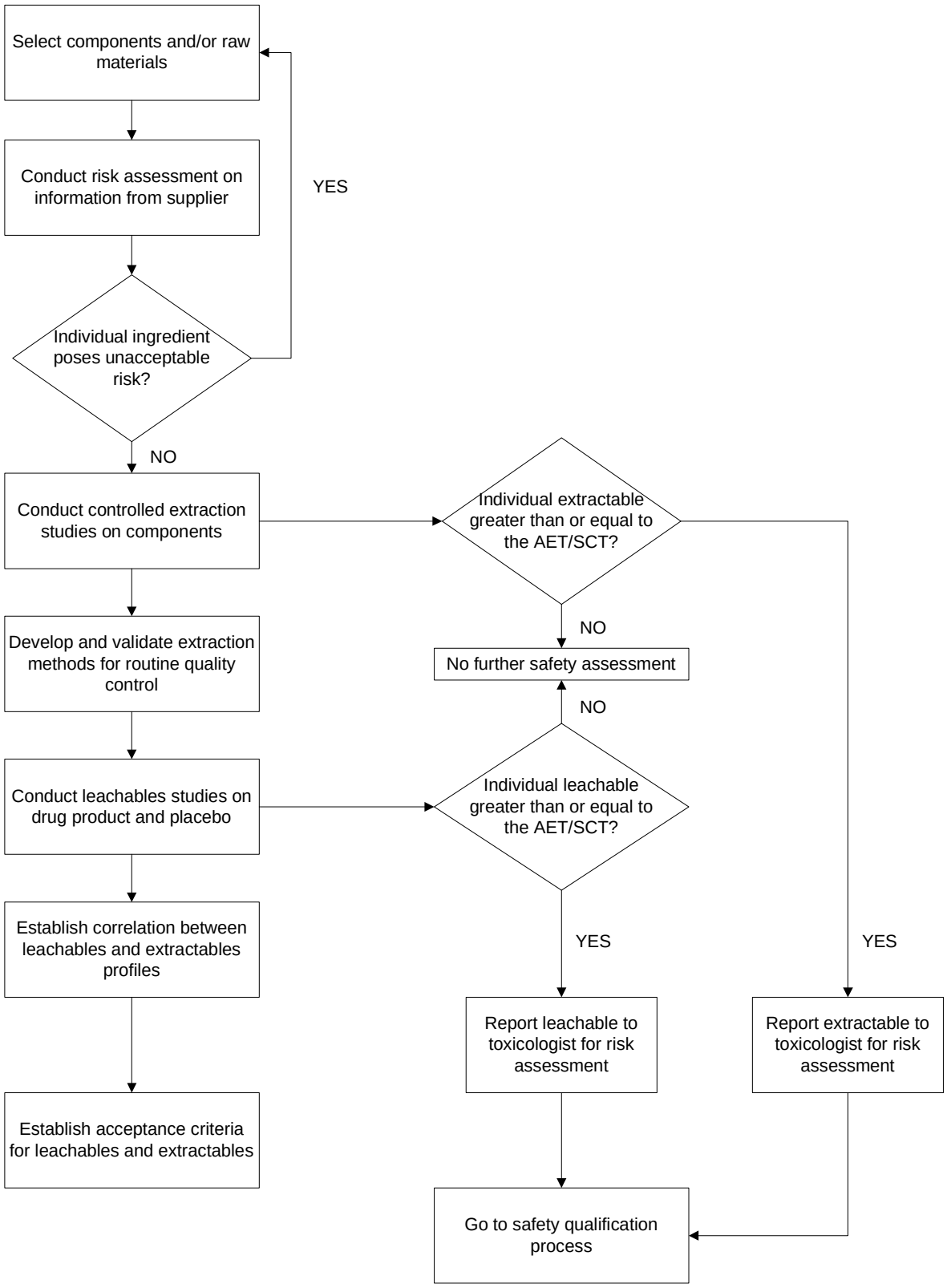
(c) The sponsor should convert the final AET from units of weight/weight to units of amount per product or dose, e.g., µg/canister, µg/dose, µg/blister, so that the AET may be applied to leachables in drug product. Any leachable at or above the final AET in units of amount per product or dose should be reported to the toxicologist for potential safety assessments. The chemist should provide adequate identification information and information on the amount of the leachable to the toxicologist. The toxicologist should clarify how much identification information is needed to conduct safety assessments.

(d) The sponsor should establish a qualitative and quantitative correlation between extractables and leachables profiles. In establishing a correlation between profiles, the results of extraction studies on multiple batches of components and leachables studies on multiple batches of drug product over multiple stability storage time-points should be examined. To establish correlations, (i) leachables profiles from multiple (at least 3) drug product definitive registration batches (e.g., NDA stability batches, bio-batches, clinical batches, toxicology study batches) using specific batches of critical components, should be compared with qualitative and quantitative extractables profiles of those specific component batches, and (ii) leachables profiles from multiple drug product registration batches should be compared with extractables profiles from multiple batches of critical components (which may not have been used in the drug product registration batches). The results of leachables studies taken from multiple stability storage time-points and conditions should be correlated with results of extraction studies. Extraction studies are conducted using multiple solvents/conditions so that asymptotic levels for extractables are achieved. Results from leachables studies should be obtained from samples incubated across the entire proposed shelf-life of the drug product, using appropriate ICH stability conditions. Extraction and leachable methods must be sufficiently sensitive to detect the full profile of extractables/leachables present above the AET, as well as be appropriately validated according to established parameters. Extractables profiles from quantitative studies should be compared with leachables profiles to determine extractables and leachables correlations. To establish a qualitative correlation between profiles, chemists must show that compounds detected in the leachables studies were also present in the Controlled Extraction Studies. To establish a quantitative correlation between profiles, chemists must show that levels of leachables obtained from leachables studies are generally less than the levels of extractables obtained from quantitative Controlled Extraction Studies.

6. Risk assessments on leachables should be performed. These should begin with structure-activity relationship (SAR) studies and thorough literature reviews, and proceed if required through toxicological evaluation studies.

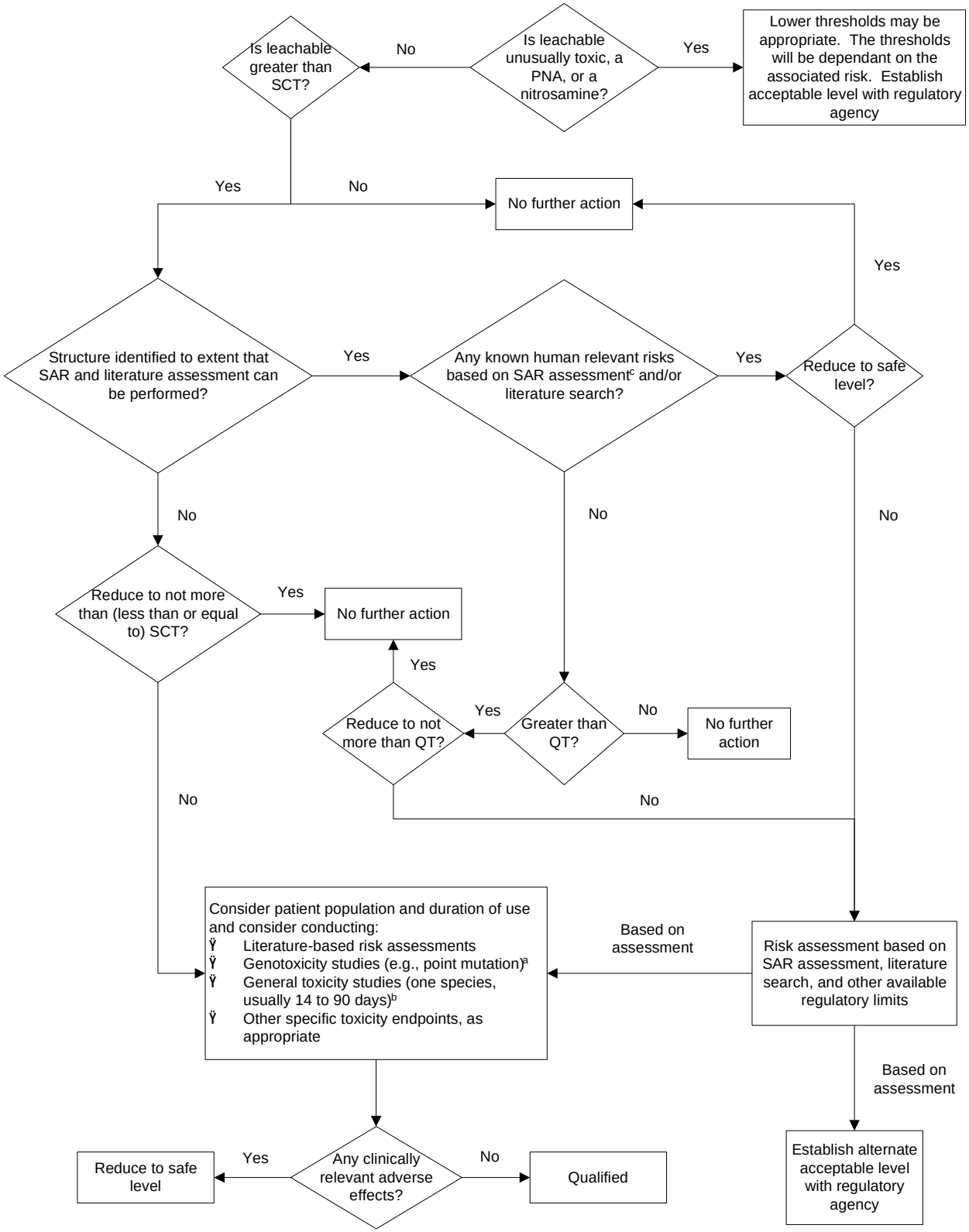
The processes summarized above are graphically displayed in flowchart form in Figures 1 and 2 below. Figure 1 depicts a pharmaceutical development process for leachables and extractables in OINDP. Note that although the performance of leachables studies and establishment of correlations and specifications is depicted as linear, often these steps are done in parallel. For example, Leachables Studies and Routine Extractables Testing of critical container/closure system components often proceed simultaneously. Additional details, guidance, and example data are contained in Part 2 and Part 3.

615 **Figure 1. Typical Pharmaceutical Development Process for L&E in OINDP**



616

Figure 2. Example Safety Qualification Process for Leachables Using Thresholds



Footnotes to Safety Qualification Process Decision Tree:

(a) If considered desirable, a minimum screen, e.g., genotoxic potential, should be conducted. A study to detect point mutations, in vitro, is considered an appropriate minimum screen.

(b) If general toxicity studies are desirable, one or more studies should be designed to allow comparison of unqualified to qualified material. The study duration should be based on available relevant information and performed in the species most likely to maximize the potential to detect the toxicity of a leachable. On a case-by-case basis, single-dose studies can be appropriate, especially for single-dose drugs. In general, a minimum duration of 14 days and a maximum duration of 90 days would be considered appropriate.

(c) For example, do known safety data for this leachable or its structural class preclude human exposure at the concentration present?

V. REFERENCES

1 Draft Guidance for Industry, Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products – Chemistry, Manufacturing, and Controls Documentation; Draft Guidance for Industry; U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER); Rockville, MD, October 1998.

2 Guidance for Industry, Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products – Chemistry, Manufacturing and Controls Documentation; Guidance for Industry; U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER); Rockville, MD, July 2002.

3 Protecting and Advancing America's Health: A Strategic Action Plan for the 21st Century, FDA, August 2003.

4 Innovation or Stagnation? -- Challenge and Opportunity on the Critical Path to New Medical Products, FDA Draft, March 2004.

5 Work Plan, PQRI Leachables and Extractables Working Group, Spring 2002.

PART 2

JUSTIFICATION OF THRESHOLDS FOR LEACHABLES IN ORALLY INHALED AND NASAL DRUG PRODUCTS

I. SUMMARY

- The *Justification of Thresholds for Leachables in Orally Inhaled and Nasal Drug Products* (the "Justification") was developed and drafted by the Product Quality Research Institute's (PQRI) Leachables and Extractables Working Group.

- In this document, the Working Group describes the development and justification of two proposed threshold values for orally inhaled and nasal drug products (OINDP): the safety concern threshold (SCT) and the qualification threshold. These thresholds were developed and justified from a toxicological (or safety) perspective, using (i) data and information from well-established databases and guidelines, and the current literature; and (ii) well-established risk assessment approaches.

- The thresholds were developed to assist in addressing part 1(a) of the Working Group's hypothesis, described in the Group's proposed Work Plan:[1]

  1. Scientifically justifiable thresholds based on the best available data and industry practices can be developed for:

     (a) the reporting and safety qualification of leachables in orally inhaled and nasal drug products, and

     (b) reporting of extractables from the critical components used in corresponding container/closure systems.

     Reporting thresholds for leachables and extractables will include associated identification and quantitation thresholds.

  2. Safety qualification of extractables, would be scientifically justified on a case-by-case basis.

- The Working Group proposes an SCT of 0.15 μg per day for carcinogens that would also provide safety for non-cancer effects, and a qualification threshold of 5 μg per day for each leachable in OINDP. Considering several marketed metered dose inhaler (MDI) products with a range of recommended doses and canister sizes, the proposed SCT corresponds to approximately 0.14 to 0.36 μg/g or 1.1 to 5.0 μg/canister. The proposed qualification threshold corresponds to 4.7 to 11.9 μg/g or 38 to 167 μg/canister.

- The SCT was developed so that it may serve as a starting point for development of an analytical threshold for leachables. This analytical threshold is called the analytical evaluation threshold (AET), and is the threshold at or above which a chemist should begin to identify a particular leachable and/or extractables and report it for potential toxicological assessment.

- The proposed qualification threshold for non-cancer effects is examined in relation to safety limits for irritants, mixtures, particulate matter in ambient air, early-life exposure (children), and compounds present in approved OINDP.

- The Working Group also proposes a decision tree for safety qualification, which utilizes both the proposed SCT and qualification threshold.

- Note that certain classes of potential leachable compounds with special safety concerns, e.g., N-nitrosamines, polynuclear aromatics (PNA's), mercaptobenzothiazole, may require much lower thresholds than proposed in this document, dedicated methods, appropriate specifications, appropriate qualifications, and risk assessments. The Working Group proposes that such leachables be considered on a case-by-case basis.

II. INTRODUCTION

The PQRI Leachables and Extractables Working Group proposes a **safety concern threshold (SCT) of 0.15 mg per day**, and a **qualification threshold of 5 mg per day** for each leachable in orally inhaled and nasal drug products (OINDP). This document provides a rationale and justification for the establishment of these thresholds for leachables in OINDP.

The document first provides an overview of the concept of leachables in OINDP and definitions of the SCT and qualification threshold for leachables. We then provide a justification of the proposed SCT, and then follow with a justification of the proposed qualification threshold.

Note that the SCT was developed to serve as a starting point for development of an analytical threshold for leachables. As shown in this document, the SCT is based on the assessment of carcinogenic data from toxicological or "safety" considerations. The Working Group recognizes that development of an analytical threshold must also include other considerations such as assessments of relevant analytical data from extractables and leachables studies. The Working Group has performed these assessments and has developed the concept of the analytical evaluation threshold (AET). The AET is defined as the threshold at or above which a chemist should begin to identify a particular leachable and/or extractables and report it for potential toxicological assessment. The AET is explained in more detail in Part 3, Chapter IV.

Furthermore, note that certain classes of potential leachable compounds with special safety concerns, e.g., N-nitrosamines, polynuclear aromatics (PNA's), mercaptobenzothiazole, may require much lower thresholds than proposed in this document, dedicated methods, appropriate specifications, appropriate qualifications, and risk assessments. Such leachables will be considered on a case-by-case basis.

The thresholds and justifications presented in this document have been developed using data and information relevant to OINDP. Therefore these thresholds should be considered applicable to OINDP and not to any other drug products. Further, these threshold recommendations are meant to provide general guidance for OINDP. The approaches used to derive the SCT are based on lifetime exposure (chronic). If a sponsor's product is for short-term use (acute), then alternative safety concern thresholds may be more appropriate, and should be discussed with the regulatory agency.

III. BACKGROUND

A. What are Leachables?

Inhalation drug products are developed for delivery of drug substance directly to the respiratory tract to treat either a local condition [e.g., asthma or chronic obstructive pulmonary disease (COPD)] or a non-respiratory disease such as diabetes. Inhaled drug substances are, by far, some of the most pharmacologically effective entities that are administered to humans -- that is they are highly efficacious at very low doses. These drugs are usually presented in delivery devices, e.g., metered dose inhalers, dry powder inhalers or nasal spray inhalers/pumps. These devices may contain polymers, elastomers, and other components from which minute quantities of material may migrate (leach) into the product and be delivered to the sensitive surfaces of the respiratory tract along with the therapeutic agent. Thus, leachables in OINDP are compounds that are present in the drug product due to leaching from container closure system components.

While every effort is taken to reduce the levels of these leachables, complete removal is not possible. For instance, a metered dose inhaler (MDI) has been demonstrated to accurately deliver relatively low doses of drug substance to the lung. However, it is also understood that the propellants employed in MDIs are reasonably good solvents and will cause a certain amount of materials to leach from the rubber-based and polymeric components in MDI delivery devices. Because these are non-drug-related impurities, there could be an increased concern for human risk by inhaling these leachates on a daily basis.

Historically, acceptable levels of leachables in a pulmonary drug product have been set by negotiation on a case-by-case basis with no standard guidelines available.

B. Potential Sources of Leachables

Leachables in inhaled drug products tend to arise from:

- Polymers
- Elastomers
- Adhesives and curing agents
- Metal components
- Dyes and pigments
- Mold release agents

During product development, careful consideration is given to the choice and rationale for selection of the components that go into the final drug product. The selection criteria are outside the detailed scope of this document. However, we recommend, wherever possible, that the materials selected comply with accepted materials for food contact or incidental food use and/or generally recognized as safe (GRAS) materials.

C. Factors Influencing Potential Dose of Inhaled Leachable

The likely patient dose of a leachable from an inhaled drug product will be related principally to the following factors:

- Concentration of leachable in the inhaler
- Number of doses taken each day

IV. GENERAL PRINCIPLES FOR THRESHOLDS

This section provides an overview of safety and analytical thresholds for leachables. It then reviews the current regulatory approaches that use thresholds to control impurities in foods and drugs, followed by an explanation of why these thresholds are inappropriate for leachables.

A. Rationale for Establishing Threshold Levels

Analytical techniques are increasingly sophisticated and capable of detecting and identifying chemicals at picogram quantities. However, it is generally accepted that there are levels of many chemicals below which the risks to human health are so negligible as to be of no consequence.

The premise of this document is that leachables present in inhalation drug products when held below data-supported threshold levels are not of concern.

Note that this document, like all current approaches to safety assessment, presents a method based on probability. This, and indeed any, safety approach cannot guarantee zero risk. This approach is in keeping with the accepted concept of safety and the current state of scientific capability, as stated clearly in Part 21 of the Code of Federal Regulations:[2]

> *Safe or safety means that there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance.*

B. Definitions of Safety Concern and Qualification Thresholds

The Working Group is proposing that the process of investigating leachable safety be based on analytical and qualification thresholds.

The analytical threshold for OINDP was determined by the Working Group through consideration of the SCT and analytical data.

806

> The **safety concern threshold (SCT)** is the threshold below which a leachable would have a dose so low as to present negligible safety concerns from carcinogenic and noncarcinogenic toxic effects.
>
> A **qualification threshold (QT)** is a threshold below which a given non-carcinogenic leachable is not considered for safety qualification (toxicological assessments) unless the leachable presents structure-activity relationship (SAR) concerns.

807

808 It is helpful to understand how the thresholds for safety concern and for toxicological
809 qualification correspond to concentrations of leachables in OINDPs. Some representative MDI
810 drug products were examined to assess this relationship. Based on 13 products with maximum
811 recommended doses of 4 to 16 actuations/day, and delivering approximately 34 to 156 mg of
812 total formulation per actuation:
813
814 • The proposed SCT of 0.15 µg/day corresponds to a range of concentrations of
815 approximately 0.14 to 0.36 µg/g or 1.1 to 5.0 µg/canister.
816
817 • Likewise, the proposed qualification threshold of 5 µg/day corresponds to a range of
818 concentrations of approximately 4.7 to 11.9 µg/g or 38 to 167 µg/canister.
819
820 We have included the spreadsheets containing these calculations in Appendix 1.
821
822
823 **C. Existing Safety Threshold Approaches**

824 To address situations in which low levels of chemical impurities pose negligible threats
825 to human health, threshold limits have been incorporated in safety assessment procedures for
826 foods and drugs. One example of this approach is the threshold of regulation for substances used
827 in food-contact articles, which is incorporated in the US food additive regulations.[3] Another
828 example is the scheme of thresholds for qualification of impurities in new drug substances[4] and
829 new drug products[5] contained in regulatory guidance developed by the ICH (International
830 Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for
831 Human Use). Both of these approaches provide quantitative threshold limits; toxicological
832 justification is not required for defined types of chemical impurities appearing in foods or drugs
833 below the threshold concentrations.
834
835 1. Food Additives

836 The threshold of regulation for substances used in food-contact articles specifies that
837 substances with no known cause for concern that may migrate into food are exempted from
838 regulation as a food additive if present at dietary concentrations at or below 0.5 parts per billion,
839 corresponding to 1.5 µg/person/day based on a total daily consumption of 3 kg of solid and
840 liquid foods.[3] The Federal Register notice publishing this regulation summarizes the scientific

justification for the threshold. The threshold was established to be low enough to ensure that exempted substances pose negligible safety concerns *even if they are ultimately shown to be carcinogenic*. Based on its analysis of the frequency distribution of carcinogenic potencies of 477 chemicals, the US Food and Drug Administration (FDA) determined that, if an exempted substance present in the diet at ≤ 1.5 µg/person/day was a carcinogen, the upper-bound lifetime risk resulting from the use of the substance is likely to be below one in a million. Because carcinogenic effects typically occur at lower levels of intake than those at which noncarcinogenic toxic effects occur,[3,6] the threshold is meant to ensure that substances that pass under it pose negligible safety concerns from noncarcinogenic toxic effects as well. The World Health Organization (WHO) has used a similar 1.5 µg/person/day threshold in the safety evaluation of certain flavoring agents, although it has not adopted this approach as an official policy.[7]

2. ICH Guidelines

ICH guidelines, Q3A(R1)[4] and Q3B(R2)[5] cover the internationally agreed principles for impurities in drug substances and products, respectively and the ICH Q3C(R3)[16] guideline covers the acceptable levels of residual solvents allowable. These guidelines have been accepted by the FDA, and have been published in the Federal Register. However, ICH Q3B(R2) addresses only those impurities in new drug products classified as degradation products of the drug substance or reaction products of the drug substance with an excipient and/or immediate container closure system (collectively referred to as *degradation products* in this guidance). *Impurities arising from excipients present in a new drug product or extracted or leached from the container closure system are not covered by this guidance*. Qualification thresholds may be based on a percentage of the active drug substance or total daily intake of the impurity. According to the guidelines, *the level of any degradation product present in a new drug product that has been adequately tested and found safe in safety and/or clinical studies is considered qualified.*

In the next section we compare the concepts of thresholds for food additives and drug impurities to those appropriate for leachables, and explain why different thresholds and approaches for establishing such thresholds are needed for leachables in OINDP.

D. Considerations for Thresholds for Leachables versus Food or Impurities

The Working Group considered the approaches and thresholds for indirect food additives and impurities in their approach to developing thresholds for leachables in OINDP. Based upon this evaluation, we propose that it is inappropriate to adopt either the threshold for food additives or impurities as a threshold for leachables, but rather we should establish new thresholds for leachables. The threshold for food additives is not appropriate for leachables because different cancer-risk levels may be appropriate for different situations, e.g., intake of drugs versus foods, and most particularly in the case of inhalation versus oral administration.

The ICH thresholds for impurities are not appropriate for leachables because:

- Unlike impurities, which are associated with the drug substance or drug product, leachables are not drug related impurities and may potentially possess different toxic characteristics. As such, analytical and qualification limits of leachable materials associated with a pulmonary product have been held to a higher standard than the approaches proposed in the ICH impurity guidelines.

- The threshold for leachables should be independent of the dose of a given drug product, as explained below.

The SCT and the qualification threshold for leachables in OINDP as well as the approach to developing this threshold are meant to be different from the ICH impurities thresholds and the ICH approach. The ICH thresholds for impurities are applied primarily, although not exclusively, to address drug related impurities. The ICH thresholds are therefore linked to the daily intake based on percentage of the active pharmaceutical ingredient, (and will vary with recommended dose).

In contrast, the proposed SCT and qualification thresholds for leachables in OINDP specifically addresses compounds leached from container/closure components, and which therefore are not derived from the drug formulation. Therefore, as described in the following pages, the Working Group developed different thresholds for leachables based on total daily intake, known toxicity data for compounds of concern, and a highly conservative risk assessment approach. Thus, even if the proposed SCT or qualification thresholds are higher than a threshold value resulting from application of the ICH standard to a particular OINDP, the proposed SCT and qualification thresholds should be considered most relevant to the given OINDP and more than adequately protective.

Furthermore, as stated previously, a threshold for leachables should be independent of the dose of a given drug product. The proposed qualification threshold for leachables in OINDP is thus independent of dose, representing a uniform value based on TDI, data and risk-assessment.

E. Thresholds for Leachables Based on Total Daily Intake

The thresholds for leachables should be expressed in terms of the total daily intake (TDI) of a leachable to which a patient would be exposed, based on the maximum daily dose of the drug product, assuming the worst case that the entire inhaled dose is delivered to the lung. This dose-related approach is similar to that used for acceptable levels of residual solvents per ICH Q3C; however, it is different from the percentage of drug approach used for acceptable levels of drug-related impurities per ICH Q3A and Q3B.

V. SAFETY CONCERN THRESHOLD

This section provides a scientific data-based rationale for a Safety Concern Threshold (SCT) of 0.15 µg/day per leachable in inhaled drug products below which a leachable need not be reported as a compound of potential safety concern. In accordance with the FDA's CMC Guidances for OINDP, the level of each leachable would be based upon the product's end of shelf-life conditions.

We first describe the decision criteria for the SCT, then provide a rationale and process for establishing the 0.15 µg/day threshold value through examination of carcinogenicity databases.

A. Decision Criteria

In general, a leachable with a TDI at or below the SCT would:

- have a dose so low as to present negligible safety concerns from noncarcinogenic toxic effects;
- be considered qualified, so no toxicological assessment would be required;
- have a low life-time cancer-risk of 1:1,000,000 (10^{-6});

For certain classes of potential leachable compounds with special safety concerns, e.g., N-nitrosamines, polynuclear aromatics (PNAs), mercaptobenzothiazole, much lower thresholds, dedicated methods, appropriate specifications and appropriate qualifications and risk assessments may be required. Such leachables will be considered on a case-by-case basis.

B. Establishment of a Safety Concern Threshold

As mentioned above in the description of the FDA threshold of regulation for indirect food additives, carcinogenic effects typically occur at lower levels of intake than those at which noncarcinogenic toxic effects occur. For example, Figure 1, shows the estimated safe human inhalation exposures for datasets of chemicals assessed for different toxicity endpoints, with the carcinogenic endpoint curve farthest to the left.

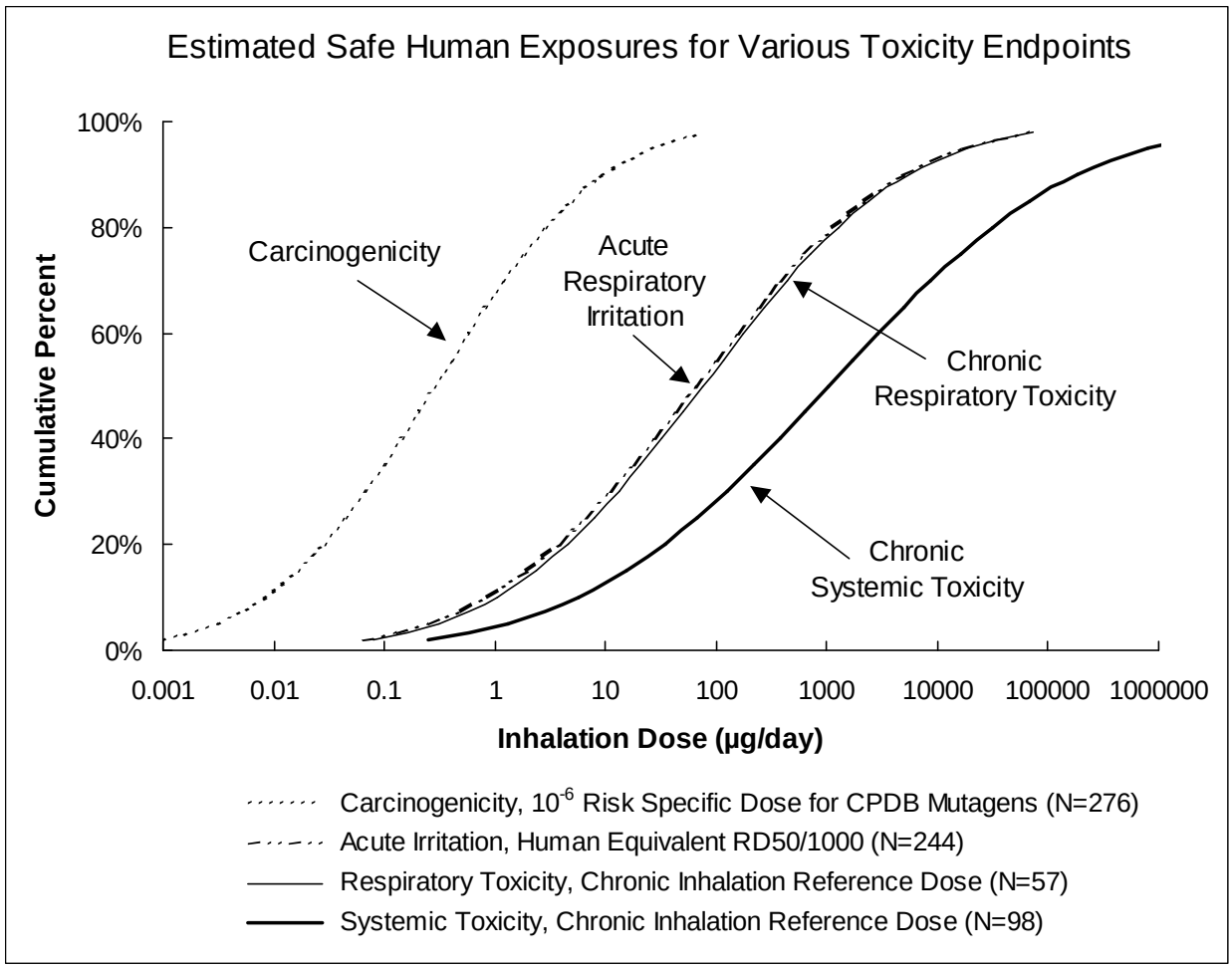


Figure 1. Cumulative distributions of estimated safe human exposures for sets of chemicals assessed for different toxicity endpoints. Cumulative percent on the vertical axis refers to the percentage of chemicals in a particular data set with an estimated safe human exposure for the indicated toxicity endpoint less than or equal to the dose on the horizontal axis. Curves shown are the log-normal curve fits for the frequency distributions. CPDB = Carcinogenic Potency Database; N = number of chemicals in each data set; RD50 = respiratory irritant dose in mice that reduces respiratory frequency by 50%.

The validity of this presumption was recently demonstrated, in the context of food additives, by an analysis of the potencies of carcinogens versus the potencies for noncarcinogenic toxicity of a wide range of compounds including highly potent chemicals exhibiting neurotoxicity, reproductive toxicity, or endocrine effects.[6] Therefore, by meeting the criterion for an acceptable cancer-risk, we will also meet the criterion for the dose being so low as to present negligible safety concerns from noncarcinogenic toxic effects. Thus, we justify the SCT based on carcinogenicity risk, using risk analysis to develop an SCT that protects human health by limiting carcinogenicity risks to an acceptable level.

First, we review definitions of key terms and concepts introduced in this section. Second, we review information collected from the databases of several health authorities that convey risk associated with certain doses of identified carcinogens. We then examine in detail the assumptions and analyses used by these authorities in developing these risk-related doses. In parallel we develop a conservative approach to identifying an SCT for OINDP, such that we have high confidence that the SCT provides the criterion for negligible safety concerns. In this approach, we use a relevant and robust subset of available data, and apply appropriately conservative assumptions reviewed in this section. Finally, we propose an SCT for OINDP, and then examine the SCT in context.

1. Terms and Concepts

Excess cancer risk is the probability or "risk" (percentage of population affected) that lifetime exposure to a carcinogen at a given dose will result in an excess cancerous effect above the background incidence. One in 100,000 (10^{-5}) and 1 in a million (10^{-6}) risk for carcinogenicity are some examples of these ratios.[8] We are particularly interested in identifying a dose associated with an acceptable cancer risk. We will therefore develop an SCT based on an appropriate "risk specific dose."

Risk specific dose is the daily dose of a particular carcinogen associated with a specified lifetime excess risk for carcinogenicity such as 10^{-5} or 10^{-6}. The daily lifetime dose associated with an excess cancer risk less than 10^{-6} is sometimes referred to as a "Virtually Safe Dose."[8] Risk specific doses are calculated from carcinogenicity "slope factors" (i.e., Risk Specific Dose = Risk Level/Slope Factor).

The **slope factor** is an estimate of the lifetime risk or probability (proportion affected) of a carcinogenic response per unit of exposure. Units are the inverse of dose rate, typically with units of $mg/(kg/day)^{-1}$. As indicated above, the slope factor can be used to estimate the dose associated with a specified risk level.

2. Review of Databases

The cumulative percent distribution of "acceptable" risk specific doses from several sources is summarized in Figure 2, in which "cumulative percent" on the vertical axis indicates the percentage of known carcinogens in a particular data set with a calculated risk specific dose less than or equal to the dose indicated on the horizontal axis. The calculation of dose in µg/day assumes a 70 kg person for all of the data sets. The median and 10^{th} percentile values from these curves are summarized in Table 1. Inhalation data are not considered separately here because there are relatively few values in these data sets that are based on inhalation data. The potency of inhaled carcinogens is addressed subsequently.

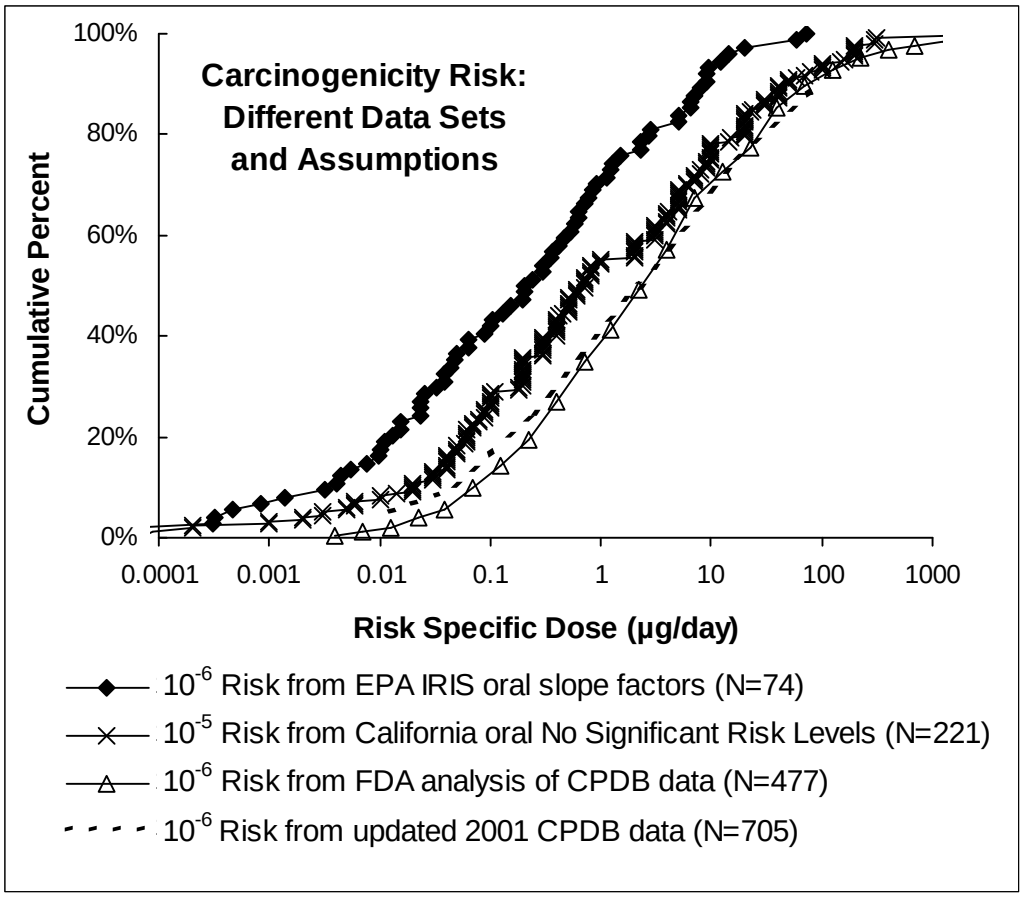


Figure 2. Distribution of acceptable cancer risk doses from different data sets.

Table 1. Summary of Risk Specific Doses From Data Sets Using Different Assumptions

Risk Level	Data Set	Route	Risk Specific Dose (µg/day)		N	Reference
			Median	10th Percentile		
10^{-6}	US EPA IRIS Slope Factors	Oral	0.22	0.004	74	[9]
10^{-5}	California NSRLs	Oral	0.70	0.020	221	[10]
10^{-6}	FDA Analysis of CPDB Data	Oral	2.33	0.047	477	[11]
10^{-6}	2001 Updated CPDB Data	Mixed	2.15	0.048	705	[12]

Abbreviations: CPDB = Carcinogenic Potency Data Base; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; IRIS = Integrated Risk Information System; NSRL = No Significant Risk Level;

3. Assumptions Used in Developing Risk-Specific Dose Values

Estimates of acceptable exposures to known or potential carcinogens vary depending on the assumptions upon which the estimates are based. The data in Figure 1 and Table 1, show that estimates of an acceptable cancer risk vary widely depending on the assumptions incorporated in the estimate. Specifically, these assumptions involve choice of an acceptable risk level, and the use of scaling factors to extrapolate from animal data to potential human risk. We explore these differences further below.

For regulatory purposes, different health authorities have used different levels of acceptable cancer risk. For example, the FDA used a one in a million (10^{-6}) level as an acceptable cancer risk in the threshold for regulation of food additives. The US Environmental Protection Agency (US EPA) also adopted a 10^{-6} level as an appropriate cancer risk for the general population in promulgating water quality criteria, and believes the target of a 10^{-6} risk level is consistent with Agency-wide practice.[13] Other health authorities, have proposed a 10^{-5} level as an acceptable cancer risk. The California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65) defines a No Significant Risk Level (NSRL) for compounds listed as carcinogens as an exposure resulting in a lifetime risk less than 1 in 100,000.[14] In a draft position paper on the limits of genotoxic impurities in medicinal products, the European Committee for Proprietary Medicinal Products (CPMP) proposes that "a lifetime excess cancer risk level of 1×10^{-5} is generally considered appropriate for defining an acceptable exposure level."[15] The ICH established a Permitted Daily Exposure for benzene and 1,2-dichloroethane as residual solvents in pharmaceutical products on the basis of a 10^{-5} carcinogenicity risk.[16] Finally, the WHO publishes guideline values for water contaminants based on 10^{-5} cancer risk but emphasizes that each country should select its own appropriate risk level.[17]

The US EPA publishes carcinogenicity slope factors for individually assessed carcinogens in the IRIS (Integrated Risk Information System) database. The US EPA typically has calculated the slope factor as the upper-bound low-dose slope (q_1*) from the linearized multistage model. Because the US EPA uses 10^{-6} as an acceptable cancer risk level, we used the US EPA oral slope factors to calculate 10^{-6} risk specific doses for carcinogens from the IRIS database. The median oral 10^{-6} risk specific dose from this IRIS data set is 0.22 µg/day (Table 1).

The California EPA calculates NSRLs using methods very similar to those used by the US EPA. However, a 10^{-5} risk is used as an acceptable level in the definition of the NSRL. Thus, the distribution of "acceptable" doses is shifted to the right compared to the 10^{-6} risk specific doses calculated from the IRIS database. The median oral NSRL is 0.7 µg/day.

Figure 1 also shows the distribution of 10^{-6} doses that was used by the FDA to establish the threshold of regulation for indirect food additives. The final regulation was based on an analysis of oral data for 477 carcinogens in the Carcinogenic Potency Database (CPDB).[18] Carcinogenic potency is expressed in the CPDB as the TD_{50}, defined as the mg/kg/day dose which will halve the probability of remaining tumor-free if administered for the standard lifespan of the species. In its analysis, the FDA approximated 10^{-6} risk specific doses by linear

extrapolation from the TD_{50} values (i.e., slope factor = $0.5/TD_{50}$). The median 10^{-6} risk specific dose in this data set is 2.3 µg/day. This distribution is shifted to the right compared to the 10^{-6} risk specific doses based on US EPA slope factors or the 10^{-5} risk level NSRLs published by the California EPA.

The FDA analysis was based on an acceptable risk level of 10^{-6}, however, unlike the US and California EPA values, the FDA analysis did not incorporate allometric scaling factors to extrapolate from rodent data. Use of the default EPA scaling factors shifts the estimated human 10^{-6} risk specific dose leftward toward lower doses by 3.76-fold for extrapolation from rats or 6.95-fold for extrapolation from mice. Another reason that might contribute to the higher estimated risk specific doses from the CPDB data is that the number of carcinogens in the CPDB is much larger than the number of compounds assessed by the US or California EPAs and may be less biased toward more potent carcinogens. Compounds evaluated by EPA may be biased toward more potent carcinogens since those compounds were presumably chosen for quantitative risk assessment based on a perceived potential for public risk. The differences in the estimates of 10^{-6} risk specific doses are probably not due primarily to the different methods for estimating carcinogenic slope factor. Based on 585 compounds from the CPDB, Krewski ***et al.***[19] demonstrated that slope factors estimated as $0.5/TD_{50}$ are similar to the q_1* estimated from the linearized multistage model, with a median value of 0.7 for the ratio of $0.5/TD_{50}$ to q_1*. Addition of new data to the CPDB has not substantially altered the distribution of carcinogenic potencies. A recent evaluation of 705 carcinogens in the CPDB, using the same assumptions as in the FDA analysis, resulted in a distribution of 10^{-6} risk specific doses (median = 2.1 µg/day) essentially identical to the original FDA analysis.[12]

4. Database Information and Assumptions Used to Develop SCT

Having reviewed the content of and assumptions used in various databases, we now identify the data and assumptions that we consider most relevant in developing the SCT. A subset of the CPDB database affords the best information.

The CPDB includes results of Ames *Salmonella* bacterial mutagenicity assays (SAL) as an indicator of genetic toxicity. This allows separate estimates of carcinogenic potency for presumed genotoxic (SAL-positive) and non-genotoxic (SAL-negative) compounds. Figure 2 summarizes the distributions of 10^{-6} risk specific doses calculated for SAL-positive and SAL-negative carcinogens in the CPDB for all routes combined (N = 454) and also from inhalation studies (N = 39). These do not include all carcinogens in the CPDB since SAL results are not available for approximately a third of them. As in the previous FDA analysis of data from the CPDB, risk specific doses are estimated by linear extrapolation from the TD_{50}. For consistency of comparison with the EPA approach, estimates are based on the most sensitive rodent species, using the default EPA allometric scaling factors, and assuming a 70 kg human.

Figure 3 shows that the SAL-positive carcinogens are, overall, about 10-fold more potent (median = 0.21 µg/day) than SAL-negative carcinogens (median = 1.9 µg/day), and therefore of greater concern. The 276 SAL-positive compounds include a substantial number (N = 37) of nitrosamines, which were not excluded. Data from the small sets of SAL-positive, and SAL-

negative inhalation carcinogens appear to follow distributions similar to those for the much larger sets of compounds from all available routes. This is consistent with an EPA analysis of 23 carcinogens for which both oral and inhalation bioassays were available. That analysis demonstrated no significant difference in carcinogenic potency between oral and inhalation routes.[20]

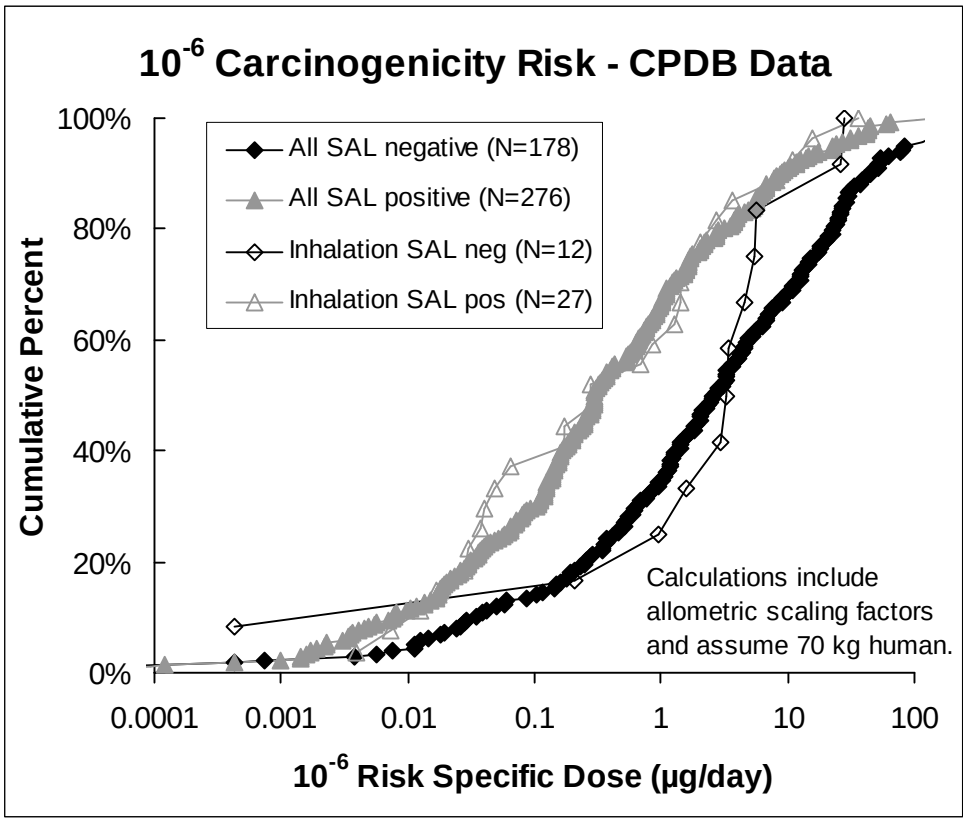


Figure 3. Carcinogenic potency of genotoxic (SAL-positive) and non-genotoxic (SAL-negative) carcinogens from the Carcinogenic Potency Data Base (CPDB).

As noted above, inclusion of an allometric scaling factor to estimate human risk specific doses has a significant effect on the calculated value. There is controversy over the application of allometric dose-scaling factors. Both the US and California EPA include scaling factors in their risk estimates. The US EPA uses default scaling factors based on body weight to the 0.75 power to represent scaling of metabolic rate across animals of different size.[21] The FDA did not include this assumption in establishing the threshold of regulation for indirect food additives. In contrast, dose metrics from rodent carcinogenicity assays are typically scaled to body surface area (body weight to the 2/3 power) on a mg/m^2 basis when reported in approved US pharmaceutical labeling.[22] In recommending drinking water standards, the WHO specifically rejected allometric scaling factors as overestimating human risk.[23] Crump *et al.* examined several metrics to express dose for 23 chemicals for which both animal and human data were

available.[24] They concluded that all dose metrics except dose rate per unit of body weight overestimated human risk. Likewise, Gaylor *et al*. concluded that the practice of estimating cancer risk based on the most sensitive rodent species-strain-sex and using interspecies scaling based on body surface area overestimates human cancer rates by about 10-fold.[25] Data in the CPDB can be construed as supporting the dose-scaling approach. For 204 carcinogens from the CPDB (including SAL-positive and SAL-negative) for which TD_{50} values are available for both mice and rats, rats are overall more sensitive when dose is expressed on a mg/kg basis. The geometric mean ratio of TD_{50}s for mice/rats is 2.6 with 95% confidence limits of 2.0 to 3.3. This dose ratio is consistent with similar carcinogenic potencies in mice and rats if dose is scaled to body surface area, and would support the use of scaling factors to estimate carcinogenic risk. Thus, there are arguments for and against including a dose-scaling factor in estimating human carcinogenic risk. If dose scaling is applied in combination with other conservative assumptions it likely that human risk will be overestimated.

Two assumptions incorporated into the derivation of carcinogenicity potency estimates deserve additional comment. Both the EPA slope factors and the FDA estimates for the threshold of regulation for food additives are based on the most sensitive rodent species. Additionally, EPA slope factors are based on the upper 95% limit on slope rather than the central estimate. Both of these conservative approaches are appropriate for estimating the potential risk for an individual regulated chemical. In that case, one wishes to be confident that an estimated risk is likely to be less than some specified level with a high degree of certainty. However, these approaches result in an overestimate of human risk when applied overall to a population of chemicals. It is extraordinarily unlikely that the actual risk for each one in a large set of chemicals would be as great as the upper 95% estimate. Likewise, apart from kinetic differences that can be addressed by dose scaling, it is also unlikely that, for every carcinogen, humans will always be at least as sensitive as the most sensitive rodent species. Thus, these assumptions are appropriate for establishing regulatory thresholds for individual chemicals but not for estimating risk parameters for a population of chemicals from a particular data set. To estimate the potency distribution for a population of carcinogens, we consider it more appropriate to use a central estimate of risk rather than the upper-bound risk estimate, and to use the geometric mean of potencies from rats and mice when both are available rather than basing the estimate on the most sensitive species.

Finally, a default human body weight of 70 kg is typically used by regulatory agencies such as the US EPA. However, a more conservative value of 50 kg is often used to calculate safety margins relative to human in US pharmaceutical labeling.[22] This 1.4-fold difference is small considering the 6 to 7 orders of magnitude range in carcinogenic potencies. Thus, an assumption of 50 versus 70 kg body weight makes relatively little difference in risk estimate, and our further calculations are based on the more protective 50 kg value.

Based on the data and issues discussed above, the subset of all SAL-positive (presumed genotoxic) carcinogens from the CPDB was chosen as the basis for estimating carcinogenicity risk to determine the SCT for OINDP. The following key points and assumptions were considered and/or applied in this choice:

- The CPDB is a large and robust database that was used previously for setting the threshold of regulation for indirect food additives.
- The SAL-positive carcinogens are more potent than SAL-negative carcinogens, and thus of greater concern.
- The slope factor approach, assuming linear extrapolation to zero risk, is more applicable to genotoxic than non-genotoxic carcinogens, for which such an assumption is questionable.
- As a basis for the SCT, the genotoxic carcinogens are especially appropriate because it is the potentially mutagenic compounds for which chemical structural "alerts" are most likely predictive, and for which structural information for a leachable is particularly desirable.
- Carcinogenic potency for the small set of carcinogens tested by the inhalation route mirrors that for the larger set of compounds tested by all routes, so that data based on all routes reported in the CPDB should be representative of the potency of inhalation carcinogens.
- The 10^{-6} level is an appropriately conservative level, and it has been used as an acceptable carcinogenicity risk by US regulatory agencies such as FDA and EPA.
- Dose scaling is an appropriate means to adjust carcinogenic potency estimates in humans for the more rapid clearance of chemicals by rodents, but combining this approach with estimates based on the most sensitive species and upper confidence limits of carcinogenic slope will likely overestimate human risk.
- The choice of 50 vs 70 kg for default human weight makes relatively little difference in risk estimate, but the more protective 50 kg value is consistent with the approach often used for US pharmaceutical labeling.

5. Identifying the SCT Value

Based on the considerations outlined above, the population of all SAL-positive mouse and rat carcinogens from the CPDB was chosen as the starting point for establishing the SCT. Human 10^{-6} risk specific doses were estimated for those compounds by linear extrapolation from the TD_{50}s, as was done previously for the FDA threshold of regulation for indirect food additives. However, the default EPA scaling factors were incorporated in the estimate, and, when data from both mice and rats was available for a particular chemical, the geometric mean of the 2 estimates of human 10^{-6} risk specific dose was used. Risk specific doses were expressed in µg/day assuming a 50 kg person. The distribution of these risk specific doses are shown in Figure 4. The median estimated human 10^{-6} risk specific dose from this data set is 0.36 µg/day.

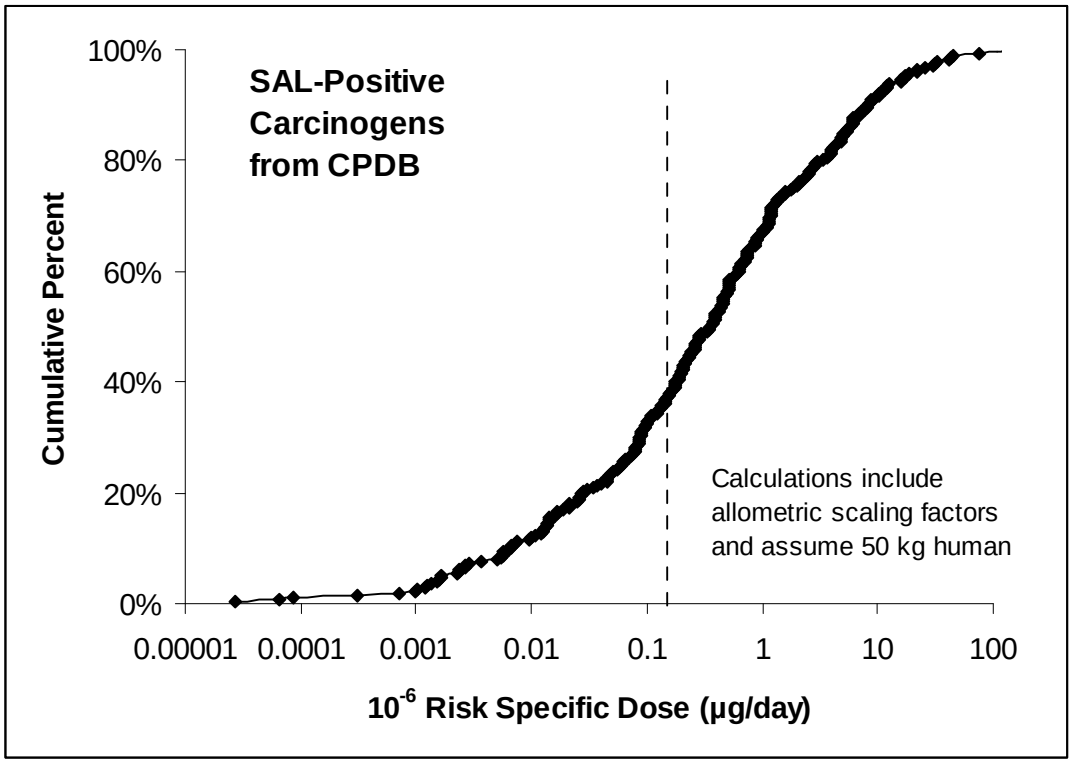


Figure 4. Distribution of estimated human 10^{-6} risk specific doses for 276 SAL-positive carcinogens from Carcinogen Potency Database (CPDB).

Based on this distribution of estimated human 10^{-6} risk specific doses for known genotoxic carcinogens, we propose a level of 0.15 µg/day as the SCT. This value of 0.15 µg/day corresponds to the 37th percentile of SAL-positive carcinogens in the CPDB. The median excess cancer risk for a SAL-positive carcinogen at 0.15 µg/day is 0. 41 x 10^{-6}. The probability that a random chemical would be a genotoxic carcinogen with a 10^{-6} risk specific dose below 0.15 µg/day is appropriately low. To estimate that probability requires both an estimate of the distribution of carcinogenic potencies (outlined above), and an assumption as to proportion of random chemicals that are likely to be carcinogens. In establishing the threshold of regulation for indirect food additives, the FDA analysis assumed that only about 20% of all chemicals are likely to be human carcinogens.[11] Coupling that same assumption, that 20% of randomly selected compounds are carcinogenic, with the 0.15 µg/day exposure level corresponding to the 37th percentile of 10^{-6} risk specific doses for known carcinogens, provides a level at which less than 10% of all compounds (20% x 37% = 7.4%) would present more than a 10^{-6} carcinogenicity risk. A recent analysis of the carcinogenic risk of chemicals concluded that less than 5-10% of all chemicals in commercial use might actually be carcinogenic in humans.[26] Thus, the assumption that 20% of chemicals are carcinogens is considered a conservative estimate.

6. The SCT in Context

Our proposed SCT of 0.15 µg/day is approximately 10-fold lower than the threshold of regulation for indirect food additives of 1.5 µg/day. The major factors accounting for the difference are that we have based our analysis only on genotoxic carcinogens and have applied dose-scaling factors in our estimates of human 10^{-6} risk specific doses. The 1.5 µg/day threshold of regulation in a 70 kg person corresponds to about the 40th percentile of 10^{-6} risk specific doses (without dose-scaling) for the 477 genotoxic and nongenotoxic CPDB oral carcinogens analyzed by the FDA.[11,27] That 40th percentile level was concluded to provide "a reasonable balance between necessary conservatism and practical utility.[11] The proposed SCT of 0.15 µg/day likewise corresponds to the 37th percentile of 276 SAL-positive carcinogens from the CPDB (with dose scaling).

Our proposed SCT equals the 0.15 µg/day threshold of toxicological concern (TTC) developed by Kroes et al.[28] for genotoxic carcinogens in the diet, assuming a cancer risk of 10^{-6} and that the genotoxic carcinogens are not N-nitroso-, azoxy-, or alflatoxin–like compounds. One small difference between this TTC and our SCT is that Kroes et al. assumed a body weight of 60 kg, whereas we are assuming a body weight of 50 kg, which is the standard weight assumed by the FDA/CDER used for labeling. In view of the magnitude of the other uncertainties in determining the SCT, we consider this small difference in the weight used in the calculations to be inconsequential.

The EMEA has adopted the TTC approach in their Draft "Guideline on the Limits of Genotoxic Impurities" for medicinal products.[15] They decided to use a cancer risk of 10^{-5} stating that this higher risk was justified by the added benefit offered by a pharmaceutical *versus* having the same genotoxic carcinogen in the diet. Additionally, the compounds in question would be drug-like compounds rather than a mixture of industrial chemical carcinogens. The EMEA's proposed TTC for genotoxic impurities is therefore 1.5 µg/day.

It is noteworthy, however, that our SCT would equal the TTC for genotoxic impurities, if the EMEA were to use a cancer risk of 10^{-6}. This equivalence between the SCT and the TTC is significant since the equivalence helps to validate our methods to develop the SCT as well as its final value.

It should be clearly understood that our approach is to establish a SCT that limits the likelihood that any individual random unidentified leachable below the threshold would present more than a 10^{-6} excess cancer risk. The SCT, by itself, is not intended to ensure an overall excess cancer risk $<10^{-6}$. For example, the threshold is not meant to ensure that a mixture of unidentified carcinogenic leachables below the threshold would result in $<10^{-6}$ overall excess cancer risk. This is consistent with the approach uniformly taken by various different regulatory agencies such as the FDA, US EPA, California EPA, WHO, and the CPMP in setting threshold levels based on carcinogenic risk. Those agencies have set threshold levels so that the risk for an individual chemical (whether identified or unknown) will not exceed some specified risk level (e.g., 10^{-6} or 10^{-5}); the thresholds have not been set to limit overall risk to those levels. For instance, a single carcinogen might be in several different consumer products at trace levels

below the California Proposition 65 NSRL, or several different carcinogens may be present in drinking water below their individual EPA-regulated levels.

A related issue should also be considered. The SCT of 0.15 µg/day limits the likelihood that a leachable below the threshold would present more than a 10^{-6} excess cancer risk. However, the average (mean) excess risk at any specified level is dominated by the small number of compounds with very high carcinogenic potencies. Thus, although the **median** excess cancer risk for a SAL-positive carcinogen at 0.15 µg/day is 0.41×10^{-6}, the **mean** excess risk for a SAL-positive carcinogen at 0.15 µg/day is about 100-fold greater (4.5×10^{-5}). However, since not all chemicals are carcinogens the mean excess risk for a random chemical at 0.15 µg/day is lower. Assuming that only 20% of chemicals are carcinogens the mean excess risk at 0.15 µg/day is approximately 8.9×10^{-6}, between the 10^{-6} and approximately the 10^{-5} levels.

Again, our approach is consistent with previous regulatory philosophy. The 1.5 µg/day threshold of regulation for indirect food additives was set by FDA to limit the probability that an indirect food additive below the threshold would be a carcinogen with an excess risk $>10^{-6}$, but was not set to ensure that the mean excess risk at 1.5 µg/day is $<10^{-6}$. We consider the best approach to protect against the influence of very potent carcinogens is not to set a much lower threshold, but to understand the types of potent carcinogens that might realistically be expected as leachables, e.g., nitrosamines and PNA's, and to employ appropriate specific thresholds and analytical methods to limit those compounds to acceptable levels.

7. Conclusions

The above considerations demonstrate the importance of having a sufficiently low SCT to allow identification of leachables with structural alerts for mutagenicity or carcinogenicity.

The distribution of potencies for SAL-positive carcinogens in the CPDB demonstrates that a SCT of 0.15 µg/day meets the criterion that a leachable with a TDI at or below the threshold is unlikely to have a life-time excess cancer-risk greater than an acceptable level of 10^{-6}.

VI. QUALIFICATION THRESHOLD

This section provides a scientific rationale, based on available data, for the toxicological qualification and acceptance of noncarcinogenic leachables in inhaled drug products using a threshold value of 5 μg TDI per leachable irrespective of patient age and disease severity. In accordance with the FDA's CMC draft and final guidances for OINDP, the level of each leachable would be based upon the product's end of shelf-life conditions.

We begin with a description of the decision criteria for the qualification threshold. We then provide a rationale and process for establishing the 5 μg threshold value through examination of reference exposure values for airborne pollutants. We then compare the significance of this threshold in the context of exposures to irritants, ambient particulate matter, marketed inhaled drug products, and mixtures. We also compare the threshold to ICH qualification thresholds, limits for early-life exposure, and thresholds for other compounds in some approved inhaled drug products.

A. Decision Criteria

In general:

- a leachable with a TDI at or below the qualification threshold would have a dose so low as to present negligible safety concerns from noncarcinogenic toxic effects;
- a leachable with a TDI at or below the qualification threshold would be considered qualified, so no toxicological assessment would be required;
- a leachable with a TDI above the SCT and at or below the QT, with a structural alert or known class effect for carcinogenicity/genotoxicity, would require a toxicology risk assessment; and
- a leachable with a TDI above the SCT and at or below the QT, with a structural alert or known class effect for immediate hypersensitivity, would require a toxicology risk assessment.

B. Establishment of a Threshold Limit (Qualification Limit)

In this section the approach to establishing a threshold limit (qualification limit) is presented. The main decision criterion for establishing the threshold was that a leachable with a TDI at or below the qualification threshold would have a dose so low as to present negligible safety concerns for noncarcinogenic toxic effects. Thus we will justify the qualification threshold based on safe exposure levels to airborne pollutants based on noncarcinogenic endpoints.

1. Databases Examined

Various United States governmental agencies have assessed the inhalation toxicity of industrial and agricultural chemicals. The US EPA, the Agency for Toxic Substances and

Disease Registry (ATSDR), and the California Environmental Protection Agency (CAL EPA) all use similar quantitative risk assessment procedures to establish **reference exposure values** considered to present a negligible risk to human health. These reference values are typically determined by applying standardized "uncertainty factors" or "safety factors" to no-observed-adverse-effect levels (NOAELs) for noncarcinogenic toxicity endpoints from animal toxicity studies or human data. (The US EPA has also recently used the benchmark dose approach to determine reference doses).

Different government agencies have developed different names for their established reference values. The reference values established by the US EPA are termed "Chronic Reference Doses" (RfDs), those established by the ATSDR are termed "Minimum Risk Levels" (MRLs), and those established by the CAL EPA are termed "Reference Exposure Levels" (RELs). Reference values established by these agencies are available in electronic databases accessible via the Internet.[9,29,30]

2. Assessment of Data

A total of 150 inhalation reference values from these databases were combined for analysis in a single data set. The toxic effect upon which the reference values were determined was a systemic toxicity endpoint for 93 chemicals, e.g., neurotoxicity, hepatic toxicity, and a respiratory toxicity endpoint for 52 chemicals, e.g., nasal or tracheal toxicity; for 5 chemicals no organ toxicity was defined at the high-dose. Reference values had been assigned by all three agencies for 18 chemicals, by two agencies for 43 chemicals, and by one agency for 89 chemicals. In those cases in which more than one reference value was available, a combined reference value was calculated as the geometric mean of the available reference values.

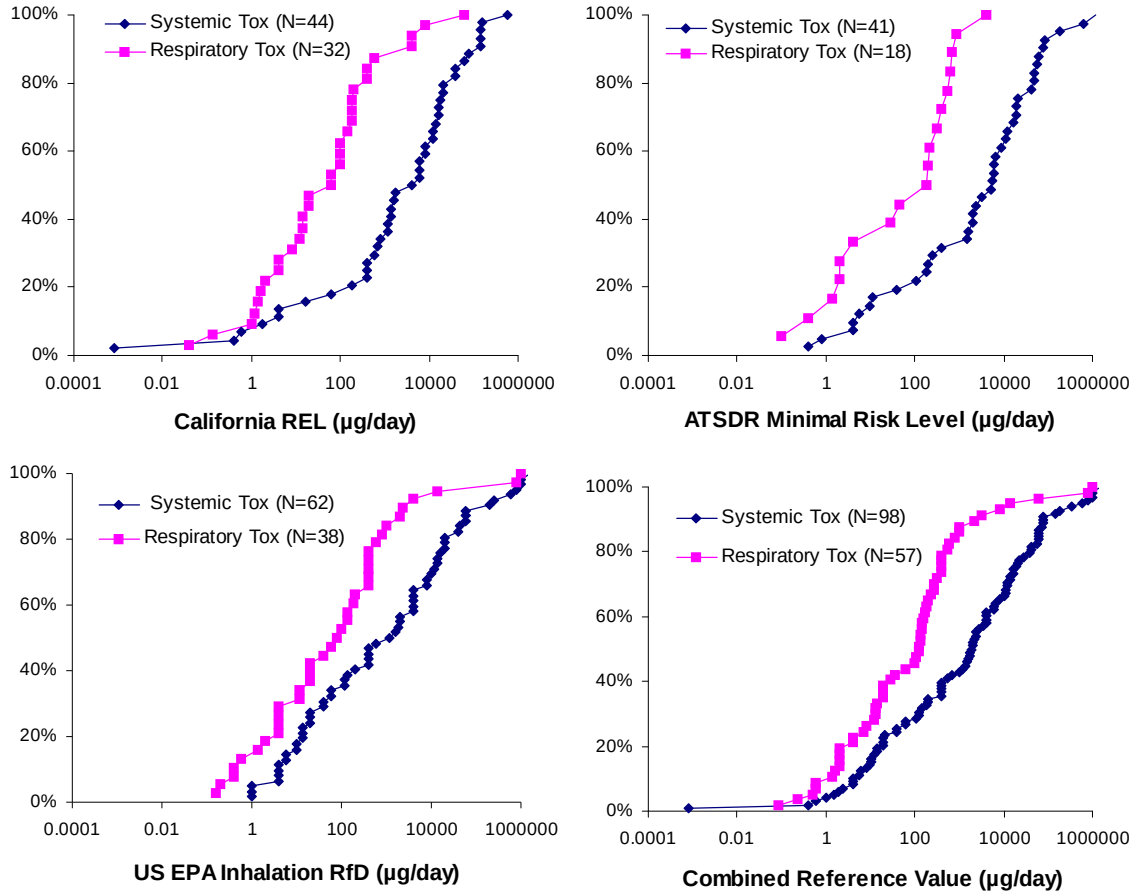


Figure 5. Distribution of Inhalation Reference Values**.** Five chemicals for which no target organ toxicity was determined were included in both the systemic toxicity and respiratory toxicity distributions.

Table 2. Summary of Inhalation Reference Toxicity Values (µg/day)

	Respiratory Toxicity		Systemic Toxicity	
	median	10^{th} %tile	median	10^{th} %tile
CAL EPA RELs	60	1.2	5000	4.0
ATSDR MRLs	189	1.1	5426	5.4
US EPA RfDs	90	0.5	1400	4.2
Combined	120	1.5	1940	5.0

The distribution of reference values for the individual and combined databases is illustrated in Figure 5. Medians and tenth percentiles for the reference values are summarized in Table 2. The median and tenth percentile reference values were similar for the three different databases. This suggests that the different agencies were dealing with sets of chemicals with similar overall toxicity, and that the different agencies used similar assumptions for extrapolating safe human exposures.

For each of the databases, median inhalation reference values were 16 to 80-fold lower for chemicals with respiratory toxicity endpoints than for chemicals with systemic toxicity endpoints. This verifies the intuitive assumption that safe inhalation doses are lower, on average, for respiratory tract toxicants than for systemic toxicants. For the combined data set, the median reference value for chemicals with respiratory toxicity endpoints was 120 µg/day, with a tenth percentile of 1.5 µg/day, and the median reference value for chemicals with systemic toxicity endpoints was 1940 µg/day, with a tenth percentile of 5.0 µg/day.

3. Identification of a Qualification Threshold

It is informative to examine the types of chemicals at the lower end of the distribution of reference values. Table 3 lists the chemicals with respiratory or systemic endpoints assigned a reference value less than 5 µg/day in any of the databases. Compounds with respiratory toxicity and inhalation reference values less than 5 µg/day are dominated by metals and metal salts, and by reactive compounds with readily identifiable irritant potential, such as aldehydes and isocyanates. For compounds with systemic toxicity, those with inhalation reference values less than 5 µg/day include metals and a variety of highly toxic compounds including dioxins and pesticides. It should also be noted that the reference values include large safety factors. For example, the reference values for acrolein employ a factor of 1000. Thus, a level of 5 µg/day is still ~100-fold less than the NOAEL level on which the reference values were based.

Overall, the data from inhalation reference values for environmental pollutants show that a qualification threshold for leachables of 5 µg TDI meets the criterion of a dose that is sufficiently low as to present negligible safety concerns for noncarcinogenic toxic effects. The inhalation reference values for most of the chemicals in the data set are well above the 5 µg/day level. Chemicals with reference values less than 5 µg/day are primarily metals, irritants, and highly toxic substances unrepresentative of the types of organic chemicals that leach from components of OINDP. Some representative compounds that may be found as leachables in an MDI are shown in Appendix 2, Table 1. Representative extractables that may be found as leachables from polymers are shown in Appendix 2, Table 2.

Since the qualification threshold has been developed using data and information relevant to OINDP, especially the inhalation reference concentrations, this threshold should be considered applicable only to OINDP and not to any other drug products.

Table 3. Chemicals from Combined Data Set with Inhalation Reference Values Below 5 µg/day

Compounds with Respiratory Toxicity			Compounds with Systemic Toxicity		
Compound	**Ref Value** (µg/day)	**Source**	**Compound**	**Ref Value** (µg/day)	**Source**
chromium vi (chromic acid mists)	0.086	REL RfD MRL	chlorinated dioxins	0.0008	REL
beryllium and compounds	0.237	RfD REL	cadmium	0.4	REL
hexamethylene diisocyanate	0.525	RfD MRL	arsenic	0.6	REL
acrolein	0.583	REL RfD MRL	arsine	1.0	RfD
chloroacetophenone, 2-	0.600	RfD	manganese	1.5	REL RfD MRL
toluene diisocyanate mixture	1.4	RfD REL	mercury	1.9	REL RfD MRL
glutaraldehyde	1.6	REL	chlordane	2.4	REL MRL
nickel & compounds	2.0	REL MRL	dicyclopentadiene	4.0	RfD
cobalt	2.0	MRL	nitroaniline, 2-	4.0	RfD
titanium tetrachloride	2.0	MRL	disulfoton	4.0	MRL
nickel oxide	2.0	REL	1,2-dibromoethane	4.0	RfD
antimony trioxide	4.0	RfD	1,2-dibromo-3-chloropropane	4.0	RfD
chlorine	4.0	RfD REL	hydrazine	4.0	REL
chlorine dioxide	4.0	RfD			
hexachlorocyclopentadiene	4.0	RfD			

Note: For compounds with more than 1 source, Ref Value is geometric mean from all available sources. Ref Value = reference value.
Information and background on REL, RfD and MRL can be found in references, 29, and 30

C. Irritants

In this section, direct respiratory tract irritation is considered. The objective is to establish a threshold below which it will be safe for anyone, including most individuals with asthma to inhale any compound and not have any substantive risk of irritation or of a bronchconstrictive asthmatic event. To do this, we examine RD50 bioassay data, which we believe to be the most relevant data for developing irritant dose limits for human populations. We then develop reference values for irritant exposure in asthmatics, based on comparative responses of normals and asthmatics to compounds in the RD50 database and other agents. We then consider the situation in which repeated exposures to a leachable could result in allergic "sensitization" and then extremely low doses could trigger an allergic or asthmatic-type reaction. Another scenario could involve an allergic asthmatic who is known to respond to extremely low levels of a compound that is present as a leachable, inhales the leachable and an asthma attack is triggered. These last two possibilities are allergic responses that could take place as an asthma attack (immediate and/or delayed hypersensitivity to an allergen) or potentially even result in a hypersensitivity pneumonitis; this is not the same as an irritant reaction. Data from isocyanates, one of the most potent known occupational allergen classes, are used to provide perspective on these scenarios. We then compare these to reference values established in other relevant databases for occupational and environmental exposure.

1. RD50 Bioassay Data Applied to Asthmatics

(a) Normals

The most well-accepted tool to evaluate sensory irritation is the study of respiratory frequency decrease in Swiss-Webster mice exposed to inhaled materials that was developed by Alarie's group. Alarie et. al.,[31] Kane et. al.,[32] and Schaper[33] have provided extensive "calibration" of this bioassay by comparing mouse to human responses. They found very similar responses between mouse and human effects in that levels producing a pronounced effect in mice, a reduction in respiratory frequency by 50%, the RD50, also showed a substantial effect in people as evidenced by burning of the eyes, nose and throat. They predicted that slight irritation would occur at 0.1 x RD50, and minimal or no effect would occur at 0.01 x RD50. This prediction of the level of minimal response in normal healthy people is supported by the high level of correlation (r^2=0.78) and near identity between industrial threshold limit values and the value of 0.03 x RD50.[33] Note that in this section, we are addressing acute irritancy, not chronic repeat dose respiratory tract toxicity. This latter subject is addressed above in section VI.B.

(b) Asthmatics Exposed to Irritants

In order to assess the effects in asthmatics, who are most likely to be the most sensitive population exposed to irritants, and to calibrate the RD50 values for application to asthmatics we have made use of the following data. Cockcroft[34] studied the distribution of responses from histamine challenge from studies in 253 normals and 181 symptomatic asthmatics. A challenge concentration of 16 mg/L produced a response in approximately 25% of the normals whereas the dose that produced the same response rate in asthmatics was approximately 0.2 mg/L. There were no observable bronchoconstrictive responses at concentrations below 0.015 mg/L, which is approximately 1/1000 of the response concentration in normals.

These data suggest that at challenge concentrations of 0.001 of those doses producing a response in normals, no observable response was seen in a large population of asthmatics. Additionally Bohm, et. al.[35] pointed out in their review that while the RD50 for toluene diisocyanate, one of the most potent respiratory irritants, is 200 ppb no effects have been reported in human epidemiology studies at concentrations below 1 ppb for mean workshift exposure. Thus a 0.001 factor appears to be well suited to compare response levels in humans and animals to non-response levels even in severe asthmatics.

In Figure 5 below, doses were calculated for which no likely acute response would be expected in the most sensitive population, asthmatics. We took the RD50 value and then calculated the inhaled dose for an adult inhaling 0.001 x the RD50 concentration for 10 minutes. The curve is labeled RD50 based estimate.

A 10 minute exposure time is assumed because this is at the low end of the animal exposure times (up to 240 min) used to generate the RD50 values. Further, even an instantaneous exposure would have an effective exposure time on the order of 10 minutes because this is the low end of the half-life for clearance of deposited materials on lung surfaces transiting into the bloodstream. Half-lives of 7-13 minutes were measured for low molecular

weight ionic species in humans[36] while half-lives are longer for most compounds.[37] Using these assumptions the expression used to calculate the inhaled threshold dose for irritation in asthmatics is:

$$\mathbf{RD50} \times \mathbf{0.001} \times \mathbf{0.14\ m^3} \qquad (1)$$

Where:

0.001 = *safety factor for asthmatics*

0.14 m^3 = *volume of air inhaled by an individual in 10 minutes in mixed activity [EPA, IRIS]*

RD50 is reported in units of ***mg/m^3***

Several studies with irritants indicate that, compared to occupational exposure limits considered to be protective for normal healthy individuals, only a relatively small additional safety factor (10 to 20-fold) needs to be used to protect asthmatics compared to normals from the potential bronchoconstrictor effects of irritants. The permissible exposure limit (PEL) for formaldehyde in the US is 0.75 ppm measured as an 8-hour time weighted average (TWA) with a 2 ppm short-term exposure limit (STEL). In asthmatics exposed to 3 ppm formaldehyde for 3 hours, there was significant eye, nose, and throat irritation but no bronchoconstriction.[38] The PEL for sulfuric acid is 1 mg/m^3. In asthmatics, 30 minutes exposure to an inhaled sulfuric acid concentration of 46 $\mu g/m^3$ (22-fold lower than the PEL) had no bronchoconstrictor effect; exposure to 130 $\mu g/m^3$ (8-fold lower than the PEL) had no statistically significant bronchoconstrictor effect although a few individual asthmatic subjects exhibited possibly meaningful bronchoconstriction.[39]

The PEL for sulfur dioxide (SO_2) is 5 ppm. In asthmatics provocative bronchoconstrictor concentrations were in the range of 0.25 to 4 ppm (20- to 1.2-fold lower than the PEL).[40]

Overall, these data suggest that the increased sensitivity of asthmatics to specific receptor-mediated bronchoconstrictors, such as methacholine, is predictive of their increased sensitivity to non-specific irritant-induced bronchoconstriction, and that there is little likelihood of bronchoconstrictor responses in asthmatics to irritants at concentrations 10 to 20-fold lower than permitted occupational exposures as defined by PELs, threshold limit values (TLVs) or short term exposure levels (STELs). A comparison of RD50s with permissible exposure levels showed that the correlation between RD50 values and TLVs is excellent when a multipler of 0.03 is applied to the RD50. Most of these exposure levels are in a similar range because they have been derived from essentially the same databases.[9] Since we have applied a multiplier of 0.001 to RD50s to arrive at exposure levels that are deemed to be safe for most asthmatics, it follows that this value is approximately 30 fold less than TLVs or PELs, and so is below the 10-20 fold value cited here as an adequate margin of safety.

2. Sensitization

Exposure to agents, such as isocyanates, that cause occupational asthma is a useful starting point for considering the possibility that repeated exposures to a leachable could result in allergic "sensitization". Data on isocyanates also are available to assess the possibility that if an individual might have pre-existing asthmatic to a leachable, what might be the result of exposure to extremely low doses of such a leachable for triggering an allergic or asthmatic-type reaction.

Studies of occupational asthma as reviewed by Karol et al[41,42] have shown that appropriate animal models can provide a close analogy of human occupational asthma. Using an inhalation model in guinea pigs it has been shown that there is a dose-response relationship to the induction of allergic reactions from occupational chemicals such as isocyanates and subtilisin and that there is also a dose-response relationship in terms of the dose that elicits an asthma-like response in sensitized individuals, *i.e,* there are practical thresholds below which allergic responses do not appear to occur.

Similar observations have been made in people with studies in people identified as having occupational asthma to isocyanates being particularly instructive. Toluene diisocyanate has a PEL of 0.02 ppm. In a study of workers exposed to toluene diisocyanate, most of those who exhibited bronchoconstriction to provocation challenge with toluene diisocyanate reacted to concentrations of 0.002 to 0.02 ppm (10-fold lower to equal to the PEL).[43] A few highly sensitive subjects reacted to very low concentrations ≤ 0.001 ppm (≥ 20-fold lower than the PEL). The data from Bohm et al showing that there were no observable cases of asthmatic response at concentrations lower than 0.001 ppm in epidemiology studies of isocyanate exposed populations also support this view.

These data support the view that the preponderance of asthmatics inhaling agents at levels of 0.001 RD50 values should be at minimal risk of developing sensitivity to inhaled chemicals or having an allergic type reaction even if they have pre-existing asthma related to the chemical. It must be recognized that there may be exquisitely sensitive individuals that can react at very low exposure levels to certain agents and it is for this reason that known allergens are treated on a case-by case basis.

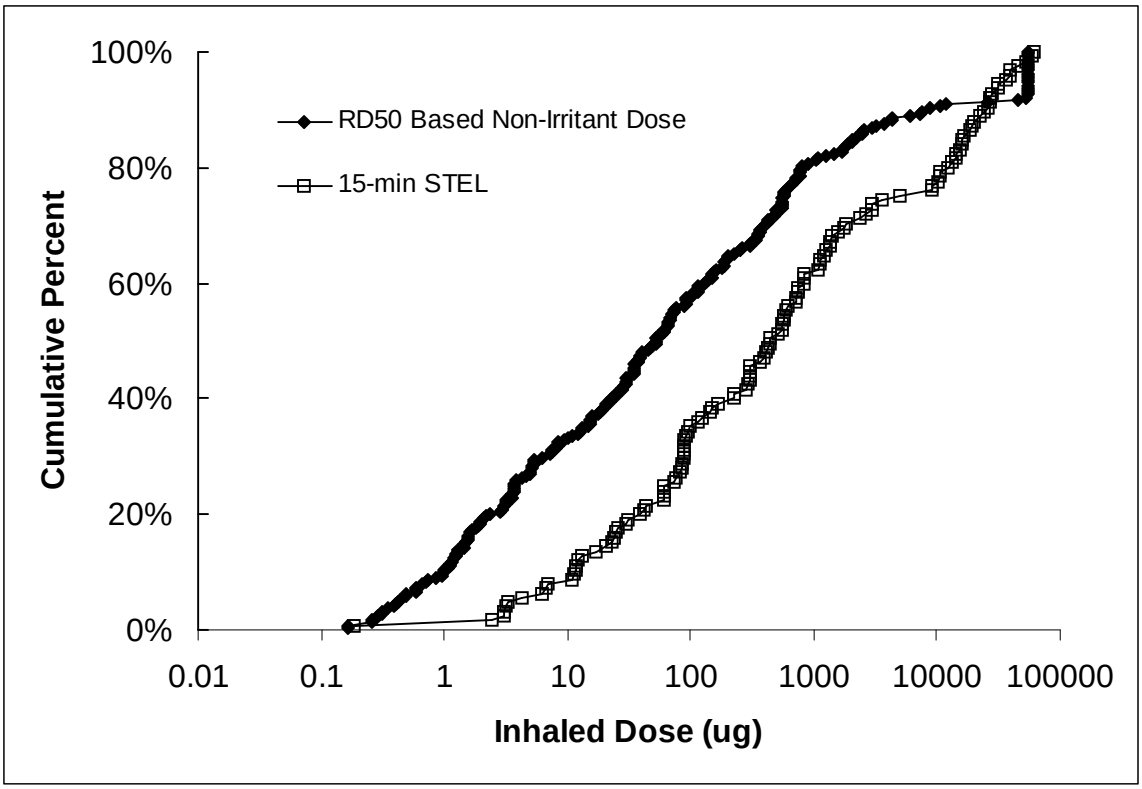


Figure 5. Comparison of cumulative percentile curves derived from RD50 based values and STEL values.

3. Comparison with Occupational STEL values

Short term exposure levels (STELS) from occupational human data (based on healthy workers) were compared to the RD50 based values. This comparison is also shown in Figure 5. STELs were chosen to compare to the RD50 data because they both are based on relative short exposures (10 minutes for RD50s; 15 minutes for STELs), which are most relevant to the short exposures involved in using medical inhalers. The STEL values include potent occupational allergens such as toluene diisocyanate at the low end of the curve and in general are applied to chemicals with a high level of concern for acute toxicity effects. As it turns out the RD50 based values are generally 10-20 fold lower than the STEL values and so these two different approaches (one based on animal data and the other on human data) provide a similar estimate of doses likely to be safe for asthmatics. The low end of the cumulative curves is comprised of a number of the most potent sensory irritants and has substantial overlap with the table on compounds with inhalation reference doses below 5 μg/day.

Only the most irritating compounds have doses that are markedly lower than the 5 μg/day qualification threshold. Approximately 27% of the 244 compounds listed in Shaper's RD50 database are below the threshold while approximately 5% of the STEL compounds are below this value. Since the 244 RD50 compounds were tested as highly likely or suspected irritants,

the database undoubtedly contains a higher percentage of irritants than a general sample of compounds such as might be found in leachables and extractables analysis.

If a compound is identified below 5 μg, structural alert information can be used to assess if it is an irritant because of the relatively small number of compounds that fall into this category, which is dominated by compounds such as isocyanates, short chain aldehydes, nitriles, and styrenes. Thus, the 5 μg/day value as a qualification threshold coupled with structural alert information to identify such compounds, is likely to be protective of irritation potential.

Compounds with structural alerts should be addressed via toxicological assessments on a case-by-case basis.

4. Comparison with California Acute REL for Irritants

The analysis in section 3 above compares the RD50 based values with data based on occupational exposure of healthy workers. Other useful data for comparison with the RD50 based values are the California Acute Reference Exposure Levels (REL),[44] which are designed to protect the general public, including sensitive subpopulations, from adverse effects resulting from a 1-hour exposure to environmental pollutants. There are acute REL for 32 chemicals that were based on irritation-related effects: 30 chemicals caused respiratory irritation, bronchoconstriction, or lower lung damage, and 2 chemicals caused eye irritation.

To compare these REL to the 10-min RD50 based values, the REL were adjusted using the modified Haber's Law equation (equation 2). Based on Haber's Law, people can be safely exposed to higher concentrations (C) of many toxins as long as the time of exposure (T) is correspondingly short:

$$\mathbf{C^n \times T = constant} \qquad (2)$$

where n is a chemical-specific parameter > 0.

The California EPA used the modified Haber's Law relationship to establish their acute REL for 1 hour using the equation:[44]

$$\mathbf{C_L^n \times T = REL^n \times 1hr} \qquad (3)$$

where C_L represents the no-observed-adverse-effect-level (NOAEL) or similar critical effect parameter from a toxicology study; and

T is the related exposure time.

For each chemical the value of ***n*** either was determined from the experimental data, when adequate data existed, or was based upon default values. For the default values, when the

toxicology study had exposure T > 1 hour, *n* was set equal to 2; for studies that had T ≤ 1 hour, *n* was set equal to 1. For our conversion of REL from 1 hour to 10 minutes, we used the same procedure, except setting *n* equal to 2 for T > 10 minutes and *n* equal to 1 for T ≤ 10 minutes.

A few of the chemicals exhibited no time dependency to their toxicity. For example, the REL for hydrogen sulfide was based upon the odor threshold, which would be time independent. In such cases, the California EPA did not use Haber's Law to make an extrapolation to 1 hour, so we did not either, using the non-adjusted acute REL instead. For all the other gases, we set *n* equal to 2 to adjust from a longer to a shorter time as was the convention of the California EPA. The equation then is:

$$\mathbf{(REL_{10\text{-}min})^n \times 10\ min = (REL)^n \times 60\ min} \quad (4)$$

The 10-min doses were calculated as

$$REL_{10\text{-}min}\ (mg/m^3) \times 0.14\ m^3 = dose \quad (5)$$

where 0.14 m^3 is the volume inhaled in 10 min.

The resultant $REL_{10\text{-}min}$ doses for the 32 chemicals calculated from Equation 5 are shown in Figure 6, along with the 10-minute RD50 based doses for comparison. It can be seen that about 16% (5/32) of the chemicals are below the 5 μg threshold. The 5 chemicals with doses under 5 μg/day are acrolein, sodium hydroxide, phosgene, hydrogen selenide, and nickel and its compounds. (*N.B. The lowest $REL_{10\text{-}min}$ dose was for acrolein, which was listed as an eye irritant, although in the toxicity study, the subjects used respirators, which prevented respiratory irritation from being assessed*).

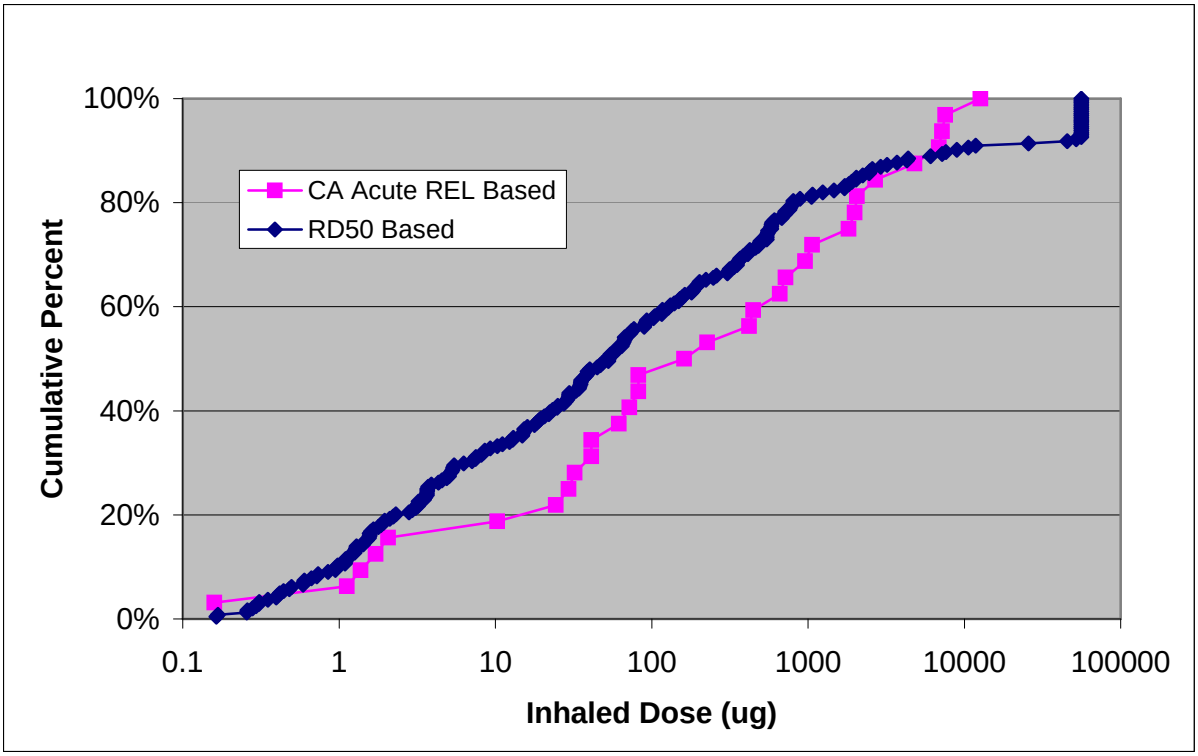


Figure 6: Comparison of cumulative percentile curves derived from California Acute REL and RD50 based values.

5. Conclusions

As demonstrated in the analysis of RD50 based values and in comparison with California Acute REL and STEL values, the proposed 5 µg TDI qualification threshold coupled with structural alert information, would adequately protect sensitive sub-populations, such as asthmatics, from exposure to irritating levels of most compounds. Compounds with structural alerts, e.g., isocyanates, short chain aldehydes, nitriles, and styrenes, should be addressed via toxicological assessments on a case-by-case basis.

D. Mixtures

The proposed qualification threshold should also offer adequate protection for mixtures of leachables. There have been relatively few studies on the toxic effects of mixtures. However, studies indicate that when chemicals having dissimilar mechanisms of toxicity are present in mixtures at concentrations far below each chemical's no observed adverse effect level (NOAEL), the chemicals typically do not exhibit additive or synergistic toxic effects. However, when these chemicals have similar mechanisms of toxicity, their effects may be additive, but not synergistic.[45,46,47,48]

E. Circumstances that May Increase Exposure to Leachables

Various circumstances can be envisioned in which patients may be exposed to higher levels of leachables than that calculated based on the recommended daily dose of a particular OINDP. For example, it is not uncommon for a patient to be taking more than one OINDP or for some patients to "abuse" their medication by taking more than the recommended daily dose on a regular basis.

In the latter case, the risk of toxicity from the known adverse effects of an overdose of the active pharmaceutical ingredient, such as a potent adrenergic agonist or corticosteroid, is likely to represent a greater safety concern than the potential toxicity from an increased intake of leachables. The appropriate regulatory approaches to this problem include educational and technical measures to decrease the likelihood of excessive use of a medication rather than adjusting the impurity limits to take inappropriate use of the product into account.

For both excessive use of one product and the use of multiple products, it is problematic to define a reasonable factor by which to adjust impurity limits to take potential increased exposure into account. Ultimately, any such approach would involve imposition of an essentially arbitrary "safety factor" to address the uncertainty in potential leachable exposure.

The Working Group considers that, given the conservative safety factors already built into the proposed qualification limit, an additional factor for potentially increased exposure is not necessary. The estimates of safe human exposure that were used to define the proposed qualification limit include large uncertainty factors, typically ≥100-fold, to take uncertainties regarding exposure and sensitivity into account.

The situation is directly analogous to the ICH approach to residual solvents. The Permitted Daily Exposures to solvents in drug products incorporate large uncertainty factors similar to those used to calculate chronic reference doses, but there is no additional specific factor taking into account the possibility that a product may be overused or a patient may be taking several drug products potentially containing the same residual solvent. Thus, the potential for increased exposure to leachables is considered to be adequately addressed by the robust nature of the proposed qualification threshold.

F. Comparison with Airborne Particulate Exposures

In this section we compare the proposed qualification threshold to levels of inhaled particulate matter. Exposure to particulates was calculated using the published average value for the level of ambient air particles in a clean reference city and estimates of daily volumes of air inspired by individuals for different ages and mixed daily activities.[49] An airborne particulate concentration of 18 $\mu g/m^3$ is used for the calculation. This value was reported by Dockery, et. al.[50] for Portage, Wisconsin, the cleanest of six cities studied intensively to establish an association between air pollution and adverse health outcomes. This was a key study used in setting the National Ambient Air Quality Standards (NAAQS) for particulate matter. Portage had the best air quality and the least cardio-respiratory disease and was therefore used as the "control" city, against which other cities were compared. For reference, as reported by Daniels

et. al.,[51] people living in the twenty largest cities in the United States would be exposed to higher concentrations of particulate matter than people in Portage. The 18 $\mu g/m^3$ value is well below the current NAAQS[52] for PM_{10} (the respirable fraction), which is set at 150 $\mu g/m^3$, twenty-four hour average, and 50 $\mu g/m^3$, annual average. (See endnote[53] below for recent information on the NAAQS standards).[54]

The calculations, summarized in Table 4, show that individuals breathing clean air with particulate concentrations below the NAAQS would be exposed to 51 to 360 µg/day of inhaled particulates. Therefore, the proposed qualification threshold of 5 µg/day represents a small percentage - between only 1% and 6% - of the quantity of particulate that these individuals are normally inhaling. These percentages would be even smaller if the comparison were being made to air concentrations of PM_{10} in major cities, or to concentrations equal to the NAAQS for PM_{10}, a value considered to be protective of public health with an ample margin of safety even, for sensitive sub-populations. For example, the mean concentration of PM_{10} during 1987-1994 in the 20 largest cities in the United States ranged from 23.8 to 46.0 micrograms/m^3.[55]

Table 4: Leachable qualification limit of 5 µg in relation to the potential daily particulates inhaled by typical healthy individuals from ambient air

Age	Body Mass (kg)	Ventilation		Inhaled Environmental Particulates *		5 µg/day Limit as % of Inhaled Environmental Particulates
		(m³/day)	(m³/kg/day)	(µg/day)	(µg/kg/day)	
1 year	11.5 †	5.1 †	0.4	93	8.0	5.4%
5 years	20.0 †	8.7 †	0.4	157	7.8	3.2%
10 years	33.7 †	15.3 †	0.5	275	8.2	1.8%
15 years	55.0 †	17.7 †	0.3	319	5.8	1.6%
Adult	58.0 †	17.8 †	0.3	320	5.5	1.6%
	70.0 ‡	20.0 ‡	0.3	360	5.1	1.4%

* Based on PM10 inhalable particle concentration of 18 mg/m³ in reference city Portage WI, USA, (Dockery et al, 1993).

† Estimates based on measurements for different ages of ventilation rate at various activity levels and percentage of daily time spent at those activity levels (Roy, 1992)

‡ Standard estimates used by US EPA for risk assessment (Reference [56])

The calculations in Table 4 are based upon daily inspired volumes for typical healthy people. Some patients may have higher daily volumes[57,58,59], but even if these volumes were doubled, the qualification limit would still represent a very small percentage of the daily dose of environmental particulate these patients would inhale – even in a city with relatively clean air.

The NAAQS for both PM_{10} and $PM_{2.5}$ are mass-based standards without regard to the chemical composition of the particulate matter. This assumes that the particulate matter on a weight basis is of equal toxicity irrespective of the chemical form, i.e., it has the same potential for causing harm.

The 5 µg per day limit, taking into consideration the risk/benefit to MDI patients, represents a minor additional load on the respiratory tract compared to the daily environmental exposure. Additionally, 5 µg is considered a worst case since, by design, it is considered a total respiratory tract burden, and does not take into account differential lung deposition, oral deposition and swallowing.

Overall, these data support the proposed qualification threshold of 5 µg TDI per leachable. A 5 µg TDI of a leachable would represent an amount of between 1 and 0.1 µg/kg/day and is between 1 and 6% of the estimated inhalable quantities of environmental particulate matter described above.

G. Comparison with Measured Polycyclic Aromatic Hydrocarbons in Ambient Air

In this section, we compare the proposed qualification threshold to estimated intakes of polycyclic aromatic hydrocarbons present in ambient air. Some compounds potentially present as leachables and extractables are routinely found in ambient air. In particular, polycyclic aromatic hydrocarbons have been measured in many communities. Eiguren-Fernandez, et. al. (2004)[60] measured vapor-phase and particle-phase content of 15 U.S. EPA priority PAHs in six communities located in urban and rural areas of Southern California over a 15-month period. Total PAH concentrations among the different communities, with the exception of the most rural community, varied between ~ 260 and ~ 607 ng/m^3. The most rural community, near the Pacific Ocean and not having any freeways, had a total PAH concentration of 68 ng/m^3. The corresponding daily intakes, assuming an inhaled volume of 20 m^3/day, are estimated to be 5.2, 12, and 1.4 µg/day. These estimated total daily intake values for PAHs provide perspective for considering the proposed safety concern threshold and qualification threshold. The SCT of 0.15 µg TDI will result in individuals potentially being exposed via the use of inhaled products to an additional quantity of PAHs that is only a small fraction of the estimated intake of PAHs from breathing ambient air. It should be recognized that polycyclic aromatic hydrocarbons have been identified in the proposal as a class of leachables to be considered on a case-by-case basis.

H. Comparison with Typical Inhaled Drug Products

In this section the proposed qualification threshold is compared to the doses for marketed inhaled drugs. We examine the significance of the 5.0 µg/day threshold for inhaled drug products by applying it to marketed MDIs and DPIs that represent a low and high range of TDI for inhaled products. Table 5 summarizes several of these marketed products, showing the maximum recommended dose of *active ingredient.*

For MDIs, we compare Serevent® and Tilade® inhalation aerosols. When taken as recommended, Serevent® can be administered for a total daily dose of 100 µg/day. Following the rationale outlined above, 5 µg of a leachable would represent 5% of the TDI. For Tilade® Inhalation Aerosol, the highest recommended TDI is 14000 µg/day. In this case, a leachable present at 5 µg would represent just 0.04% of the TDI.

For DPIs, we examine Foradil® and Relenza®. The highest recommended TDI for Foradil® is 20 µg/day. As such, 5 µg of a leachable would represent 25% of the TDI. For Relenza®, which has a recommended daily intake of 20,000 µg/day, 5 µg of a leachable represents 0.025% of the TDI.

I. Comparison with Accepted Levels of Leachables

The proposed safety concern and qualification thresholds were compared to accepted levels of a representative and blinded list of leachables (n = 82) that were present and evaluated for safety in approved inhalation drug products.[61] Of note, the accepted levels were not necessarily set based upon safety considerations but often represent observed levels that were below potentially acceptable levels based on safety data. Eleven compounds (13%) were present at daily exposure levels below the SCT of 0.15 µg/day; compounds of particular concern in this group include nitrosamines and PAHs. An additional 37 compounds were associated with accepted levels that were equal to or below the QT of 5 µg/day but greater than the SCT; the daily exposure levels of the remainder of the listed compounds exceeded the QT. Thus, 58% (48/82) of compounds on this list were present below the proposed qualification threshold of 5 µg/day and would need to be evaluated only if they presented special concern, such as a structural alert for mutagenicity or irritant activity. Therefore, the current experience with leachables in OINDP suggests that the proposed thresholds provide practical decision making criteria for use in safety evaluation of leachables for general toxicologic, mutagenic/carcinogenic, and sensitization potential.

J. Comparison with ICH Impurity Guidelines

ICH guidelines Q3A and Q3B provide qualification thresholds for process and drug-related impurities in drug substances and products. Table 5 illustrates the range of thresholds, in terms of µg/person/day, for qualification of impurities at the recommended dose levels for some representative inhalation drug products. For drug substance impurities, the qualification thresholds range from 0.03 to 60 µg/day with a median value of 1.9 µg/day. For drug product impurities, the qualification thresholds range from 0.2 to 200 µg/day, with a median value of 12.9 µg/day.

The proposed threshold of 5 µg/day for qualification of leachables in OINDP is intermediate between these values, less restrictive than applying the criteria for impurities in new drug substances but more cautious than applying the criteria for impurities in new drug products.

Note that the 5 µg/day qualification threshold for leachables in OINDP, as well as the approach to developing this threshold, are meant to be different from the ICH impurities thresholds and the ICH approach. The ICH thresholds for impurities are applied primarily, although not exclusively, to specifically address drug related impurities. The ICH thresholds are therefore linked to the daily intake based on percentage of the active pharmaceutical ingredient, (and will vary with recommended dose).

In contrast, the proposed qualification threshold for leachables in OINDP specifically addresses compounds leached from container/closure components, and which therefore are not derived from the drug formulation. As highlighted in Part IV, Section D, leachables are not drug related impurities and may possess much different toxicity characteristics. Therefore, the Working Group developed a different threshold for leachables based on total daily intake, known toxicity data for compounds of concern, and a highly conservative risk assessment approach. Thus, even if the proposed 5 µg/day qualification threshold is higher than a threshold value resulting from application of the ICH standard to a particular OINDP, the 5 µg/day qualification threshold should be considered most relevant to the given OINDP and more than adequately protective.

Furthermore, a threshold for leachables should not be dependent on the dose of a given drug product. The proposed qualification threshold for leachables in OINDP is thus independent of dose, representing a uniform value based on TDI, data and risk-assessment.

Table 5. ICH Thresholds for Qualification of Drug-Related Impurities in Some OINDPs

Product	Type	Active Ingredient	Maximum Dose * (µg/day)	ICH Qualification Threshold			
				Drug Substance		Drug Product	
				basis	(µg/day)	basis	(µg/day)
FORADIL	DPI	formoterol fumarate	20	0.15%	0.03	1%	0.2
SEREVENT	MDI-DPI	salmeterol xinafoate	100	0.15%	0.15	1%	1.0
FLONASE	NAS	fluticasone propionate	200	0.15%	0.30	1%	2.0
ATROVENT	MDI	ipratropium bromide	216	0.15%	0.32	1%	2.2
ATROVENT	NAS	ipratropium bromide	252	0.15%	0.38	1%	2.5
BECONASE AQ	NAS	beclomethasone dipropionate	336	0.15%	0.50	1%	3.4
QVAR	MDI	beclomethasone dipropionate	512	0.15%	0.77	1%	5.1
ASTELIN	NAS	azelastine hydrochloride	1,096	0.15%	1.6	1%	11.0
VANCERIL 84	MDI	beclomethasone dipropionate	1,260	0.15%	1.9	1%	12.6
PULMICORT	DPI	budesonide	1,280	0.15%	1.9	1%	12.8
PROVENTIL HFA	MDI	albuterol sulfate	1,296	0.15%	1.9	1%	13.0
AZMACORT	MDI	triamcinolone acetonide	1,600	0.15%	2.4	1%	16.0
FLOVENT	MDI-DPI	fluticasone propionate	2,000	0.15%	3.0	1%	20.0
AEROBID	MDI	flunisolide	2,000	0.15%	3.0	1%	20.0
MAXAIR	MDI	pirbuterol acetate	2,400	0.15%	3.6	1%	24.0
INTAL	MDI	cromolyn sodium	6,400	0.15%	9.6	≤50 µg	50.0
TILADE	MDI	nedocromil sodium	14,000	0.15%	21	0.50%	70.0
RELENZA	DPI	zanamivir	20,000	0.15%	30	0.50%	100
IMITREX	NAS	sumatriptan	40,000	0.15%	60	0.50%	200
NICOTROL NS	NAS	nicotine	40,000	0.15%	60	0.50%	200
Median			1,448		1.9		12.9

* Based on dose delivered from mouthpiece or actuator when reported. "Every 4 hours" is assumed to allow up to 6 times daily.

Abbreviations: DPI = dry powder inhaler; MDI = metered dose inhaler; NAS = nasal spray

K. Children

1. Qualification Threshold and Protection against Non-carcinogenic Leachables

Studies to date support that the qualification threshold adequately protects children from the toxic effects of leachables that are non-carcinogenic. In this section, we will describe the current scientific database that leads us to this conclusion.

Children are a concern since they may have increased sensitivity to toxicants. We would expect that children are adequately protected since the qualification threshold is based upon inhalation references values that are intended to protect essentially all people, including sensitive subpopulations such as children. By understanding the process for setting these reference values, we can better determine that children are in fact protected. We will focus on the EPA's process since this has been adopted by the California EPA and ATSDR (see Figure 4). The following is a description of the EPA's process by Dourson and coworkers,[62] which also addresses protection for children.

When establishing RfD and RfC values, the EPA identifies the NOEAL, lowest-observed-adverse-effect-level (LOAEL), or benchmark dose or concentration and then divides this value by a series of uncertainty factors, two of which are relevant to assessing children's risk. One uncertainty factor accounts for the completeness or incompleteness of the toxicity dataset for the reference value. A complete dataset would include investigations of the chemical's toxicity over most of the test animal's life stages. Examples of incomplete datasets would be missing developmental and reproductive toxicity studies, including tests on younger animals. When such incomplete datasets are used, and it is suspected that developmental or reproductive toxicity could occur at doses below the identified NOAEL, then the EPA includes an uncertainty factor of 3 or more commonly 10. These values have been justified using a dataset of 69 pesticides for which extensive toxicity data exists, and comparing the NOAELs for chronic toxicity with those for developmental and reproductive toxicity.[63]

The other uncertainty factor relevant to children accounts for variability in toxic response among people, including highly sensitive subjects, such as children and elderly. This intraspecies uncertainty factor usually has a value of 10. This factor can be equally divided into a toxicokinetic variability component with a default value of 3.16 [i.e., $(10)^{1/2}$], and a toxicodynamic variability component also with a default value of 3.16, assuming these components act independently.

The intraspecies uncertainly factor of 10 and the associated subfactors of 3.16 have been justified for children based upon multiple studies that have compared the clinical response to pharmaceutical agents in children versus adults as well as the toxic response to chemical agents in younger versus older animals.[64,65] For example, the National Academy of Sciences Committee on Pesticides in the Diets of Infants and Children reviewed several human and animal studies and concluded that the 10-fold intraspecies uncertainty factor was sufficient to protect infants and children.[66] Renwick and Lazarus analyzed the toxicokinetic data of 60 xenobiotics and the toxicodynamic data of 49 xenobiotics in adults, children, and other groups.[67] They concluded that the composite 10-fold factor covered the great majority of the population

(>99.9%), including children. Renwick compared the toxicokinetics, i.e., clearance and elimination half-lives, of 22 drugs in infants and children in relation to adults.[68] For 20 (91%) of the drugs, the differences in elimination between children and adults were small enough to be covered by the default 3.16-fold toxicokinetic variability factor.

All of the above data were for exposures to xenobiotics via non-inhaled routes. One study by Pelekis and colleagues[69] has addressed whether the uncertainty factor is adequate for children exposed via inhalation. They used physiologically based pharmacokinetic models to compute the toxicokinetic variability factors for adults and children who were exposed to volatile organic gases. For the computed pharmacokinetic parameters for each gas, the variability was small enough to be covered by the default 3.16-fold toxicokinetic variability factor. To our knowledge, no study has been conducted to justify the intraspecies uncertainty factor of 10 or the toxicodynamic factor of 3.16 for inhalation exposures of adults or children.

Further work is needed to determine whether the default uncertainty factors offer adequate protection for children, especially for exposure to gases and particles. In comparison to adults, children generally have higher ventilation on a body weight basis,[70] and higher total and regional deposition of particles in the lung[71,72,73,74] resulting in higher deposited doses, especially per unit surface area,[75] and thus increased likelihood of toxicity.

Most xenobiotic metabolic enzyme systems in the body are fully developed by 6 months postnatal, and more assuredly by 1 year.[76] However, the xenobiotic metabolic systems in the lung may take longer to fully develop. For example, the cytochrome P450 monooxygenase system develops in tandem the maturation of the Clara cells and endothelial cells in lung parenchyma.[77] Studies in humans indicate that it may take longer than 6 months to a year for Clara cells to differentiate fully.[78] While these metabolic systems are developing, children will be more sensitive than adults to the toxic effects of many, but not all, xenobiotics.[79] However once the metabolic systems are fully developed, the sensitivity of children tends to be the same as adults on a body weight basis.[79]

2. Safety Concern Threshold and Protection against Carcinogenic Leachables

We now turn our attention to the safety concern threshold and whether this adequately protects children from leachables that are carcinogens. The available data indicate the SCT provides adequate protection for many potential carcinogens that may be in OINDP, but would not have special safety concerns, e.g., nitrosamines, PNA's. The basis for making this conclusion is the EPA's *Supplemental Guidance for Assessing Cancer Susceptibility from Early-Life Exposure to Carcinogens*.[80] In this guidance, the EPA makes a distinction between the cancer risk of carcinogens acting via mutagenic versus non-mutagenic modes of action. The EPA concludes that children who are exposed to mutagenic carcinogens between age 0 (birth) and <16 years have an increased cancer risk over a 70-year lifetime, with the risk being higher for early childhood exposures. However, for children who are exposed to non-mutagenic carcinogens, the EPA concludes that the current data are insufficient to assess whether these exposures would result in an increased lifetime cancer risk.

To compute the increased risk for children exposed to mutagenic carcinogens, the EPA

proposes the cancer slope factor be increased by 10-fold for exposures before 2 years of age (i.e., 0 to <2 yrs) and 3-fold for exposures between 2 and <16 years of age. For exposures after turning age 16, no further adjustment is needed. For exposures that continue fairly uniformly over a lifetime, the EPA acknowledges the resultant increases in cancer risk are relatively small, especially when compared to the total uncertainty in the estimates themselves. For children continually exposed to a uniform level of a mutagenic carcinogen from age 0 to <16 years and from age 2 to <16 years, the estimated lifetime cancer risk would increase by 1.63-fold and 1.34-fold, respectively.

To assess how these adjustments relate to the cancer risk of children exposed to leachables from OINDP, it should be noted that the SCT does not apply to any leachable compounds with special safety concerns, e.g., nitrosamines and PNA's, which are all mutagenic carcinogens and instead would be addressed on a case-by-case basis. Note that mercaptobenzothiozole is a carcinogen but not a mutagen. Therefore, the only scenario for which the SCT would not offer sufficient protection to children would be for a leachable that is a mutagenic carcinogen but not categorized as having special safety concerns. In this scenario, one would need to consider if the SCT can be appropriately applied.

3. Conclusions

Based on the limited data available, the qualification threshold appears to protect children from the toxic effects of leachables that are noncarcinogenic. There are data showing that the default toxicokinetic variability factor does protect children for inhaled gases. However more research is needed to determine whether the default toxicodynamic and intraspecies uncertainty factors offer adequate protection for children, especially for exposure to gases and particles.

Similarly, the safety concern threshold should protect children from leachables that are non-mutagenic carcinogens. Mutagenic carcinogens with special safety concerns would not use the SCT, and instead would be addressed on a case-by-case basis. The SCT is considered more than adequate to protect children from carcinogens in all but the most unusual and specific circumstances.

L. Other Considerations

The 5 μg/day (< 1 μg/kg) threshold for a leachable in an inhaled drug product can be further put into perspective by considering other compounds in some approved inhaled drug products.

The proposed FDA specifications for the alternative propellant HFA 134a, include limits of 5 ppm for "total unsaturates"[81] in the propellant. Unsaturated compounds are highly reactive species and a patient could easily receive 16 actuations a day (4 doses of a steroid, 4 of a long acting β_2-agonist and 8 actuations or more of a rescue medication). Under these circumstances the patient could inhale 8 μg of an unsaturated compound, which is more than the proposed leachable threshold.

Another example can be drawn from a typical valve leachable, 2,2'-methylenebis(4-

methyl-6-tertbutylphenol) (CAS 119-47-1), which is present in some MDI formulations. Takagi *et. al.,*[82] quoted a no-effect oral dose of 0.03% in the diet during chronic preclinical studies of up to 18 months duration. This represented doses of approximately 18 mg/kg/day. Applying the Agency's safety factors of 1000[83] an acceptable daily intake would be equivalent to NOEL/1000 or 0.018 mg/kg/day some 18 times greater than the proposed threshold.

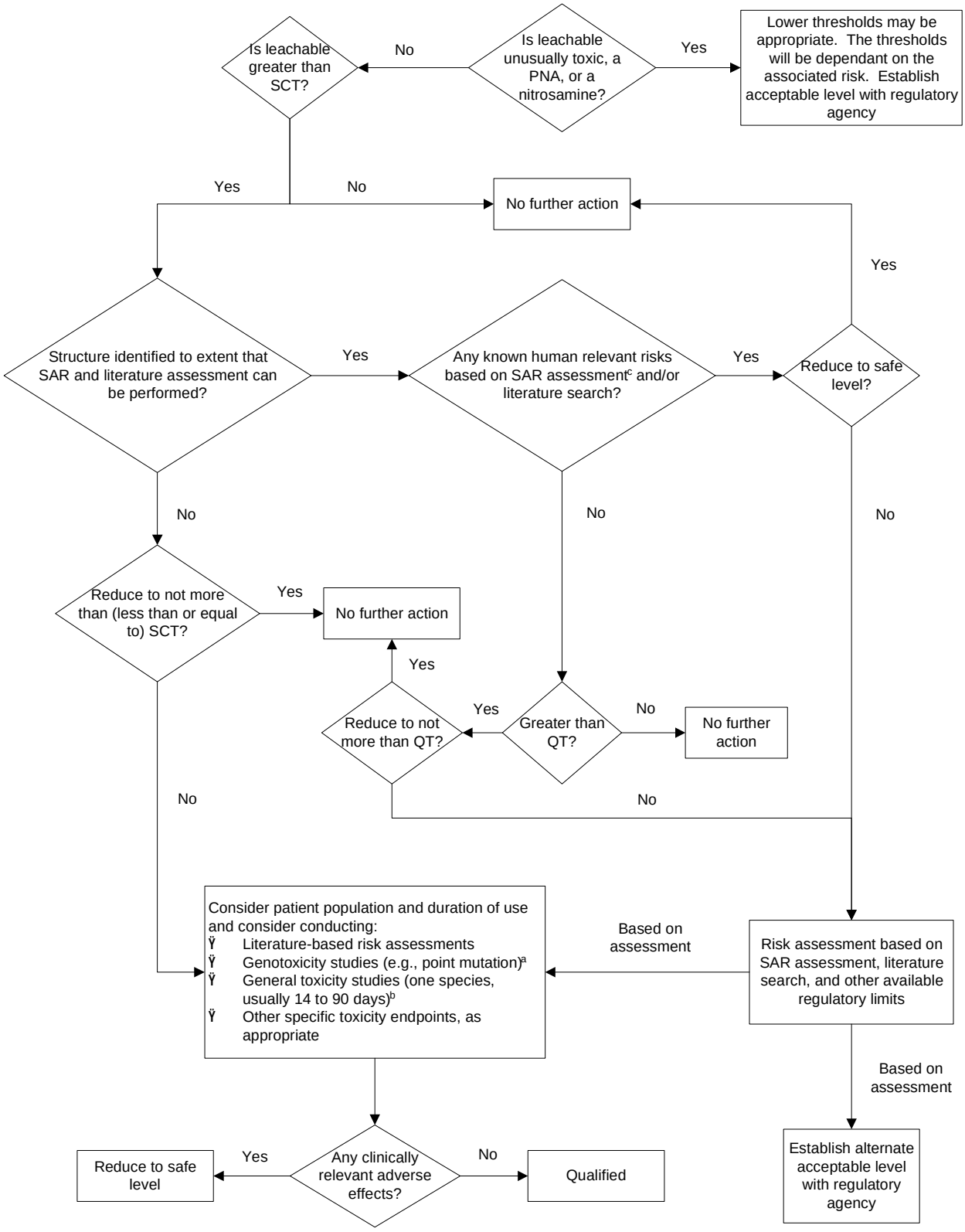
VII. SAFETY QUALIFICATION PROCESS USING THRESHOLDS

In general, the rationale for the process of how to qualify leachables in OINDP follows a similar strategy employed in ICH Q3A and Q3B, respectively.

If data are unavailable to qualify the proposed acceptance criterion of a leachable, studies to obtain such data can be appropriate when the safety concern and qualification thresholds for leachables in OINDP are exceeded. Higher or lower thresholds for qualification of leachables can be appropriate for some individual OINDP based on scientific rationale and level of concern. Proposals for alternative thresholds would be considered on a case-by-case basis. As previously indicated, for certain classes of potential leachable compounds with special safety concerns [nitrosamines, polynuclear aromatics (PNA's), mercaptobenzothiazole], much lower thresholds, dedicated methods, appropriate specifications and appropriate qualifications and risk assessments may be required. Such leachables will be considered on a case-by-case basis.

The “Decision Tree for Identification and Qualification” (below) describes considerations for the qualification of leachables when thresholds are exceeded. In some cases, decreasing the level of a leachable to not more than the threshold can be simpler than providing safety data. Alternatively, adequate data could be available in the scientific literature to qualify a leachable. If neither is the case, additional safety testing should be considered. The studies considered appropriate to qualify a leachable will depend on a number of factors, including the patient population, daily dose, and duration of drug administration. Such studies can be conducted on the OINDP containing the leachables to be controlled, although studies using isolated leachables can sometimes be appropriate.

A. Decision Tree for Identification and Qualification



Footnotes to Safety Qualification Decision Tree

(a) If considered desirable, a minimum screen, e.g., genotoxic potential, should be conducted. A study to detect point mutations, in vitro, is considered an appropriate minimum screen.

(b) If general toxicity studies are desirable, one or more studies should be designed to allow comparison of unqualified to qualified material. The study duration should be based on available relevant information and performed in the species most likely to maximize the potential to detect the toxicity of a leachable. On a case-by-case basis, single-dose studies can be appropriate, especially for single-dose drugs. In general, a minimum duration of 14 days and a maximum duration of 90 days would be considered appropriate.

(c) For example, do known safety data for this leachable or its structural class preclude human exposure at the concentration present?

B. USP and ISO Standards

Note that for pulmonary drug products, United States Pharmacopoeia (USP) <87> and <88>, and ISO 10993 may be appropriate for suppliers of OINDP device components but not necessary for drug product manufacturers. Drug product manufacturers need not perform these tests when a more comprehensive in-vivo toxicological evaluation is available.

VIII. CONCLUSIONS

The information provided in this Part provides a scientific rationale to establish a SCT of 0.15 µg and a qualification limit of 5 µg per leachable for TDI from individual inhalable drug products.

Based on the information provided in this technical review:

- The current FDA threshold for regulation for substances used in food-contact articles is considered inappropriate for leachables.
- The current ICH guideline (Q3B) for impurities and degradants in drug product is considered inappropriate for leachables.
- A 0.15 µg TDI SCT for a leachable should be considered as a starting point for development of an analytical threshold that will adequately protect the safety of patients from both carcinogenic and noncarcinogenic toxic effects.
- A 5 µg TDI limit for qualification of a leachable will adequately protect the safety of patients from noncarcinogenic toxic effects.
- The thresholds and justifications presented in this document have been developed using data and information relevant to OINDP. Therefore these thresholds should be considered applicable only to OINDP and not to any other drug products.

The weight of scientific evidence strongly supports the use of a 0.15 µg TDI safety concern threshold and a 5 µg TDI qualification threshold for noncarcinogenic leachables associated with inhaled pharmaceutical products. Establishment of a 5 µg TDI qualification threshold will allow preclinical evaluations to focus on substantive issues related to product safety and avoid evaluation of trace leachables unless structural information indicates a basis for further evaluation. This strategy provides a high level of assurance that these products are safe for patient use.

IX. GLOSSARY

ATSDR	Agency for Toxic Substances and Disease Registry
CAL EPA	California Environmental Protection Agency
Cancer-risk ratio	Ratio that conveys the probability or "risk" that lifetime exposure to a carcinogen at a given dose will result in an excess cancerous effect above the background incidence. 1 in 100,000 (10^{-5}) risk for carcinogenicity and 1 in a million (10^{-6}) risk for carcinogenicity are some examples.
Cumulative Percent	The percentage of cases falling below a specified value within a distribution of values; can be used interchangeably with "percentile."
Dose (for inhalation and nasal spray products)	The amount of drug delivered after actuating the inhaler (or spray) the minimum number of times specified on the label.
EPA	United States Environmental Protection Agency
FDA	United States Food and Drug Administration
ICH	International Conference on Harmonisation
ISO	International Standards Organization
Linearized Multistage Model	Dose-response model which assumes that the dose-response function for carcinogenicity is unlikely to exceed linearity in the low dose region. Used with data that includes only the number of animals with cancer. Expresses upper confidence limits of cancer risk as a linear function of dose.
MRL	Minimum risk levels. MRLs are reference values established by the ATSDR
PQRI	Product Quality Research Institute
Qualification	Examination of data from testing, e.g., toxicology data, literature data, structure-activity relationship data, clinical safety experience, regarding given leachable compound, with acceptable risk assessment.
Qualification Threshold	The threshold below which a given leachable is not considered for safety qualification (toxicological

	assessments) unless the leachable presents structure-activity relationship (SAR) concerns.
RD50	Exposure concentration that causes a 50 % reduction of respiration rate in mice, due to sensory irritation.
RfD	Chronic reference doses. RfDs are reference values established by the EPA.
Reference Values	Dose values associated with given compounds, which are considered to present a negligible risk to human health. Usually established via risk assessment methods.
REL	Reference exposure levels. RELs are reference values established by the CAL EPA.
Risk Specific Dose	The daily dose of a particular carcinogen associated with a specified lifetime excess risk for carcinogenicity, such as 10^{-5} or 10^{-6}.
Safety Concern Threshold (SCT)	The threshold below which a leachable would have a dose so low as to present negligible safety concerns from carcinogenic and noncarcinogenic toxic effects.
Slope Factor	An upper-bound estimate of the lifetime risk or probability (proportion affected) of a response per unit of exposure. Units are in $mg/(kg/day)^{-1}$. For carcinogens, the slope factor is used to estimate an upper-bound probability of an individual developing cancer as a result of a lifetime of exposure to a particular level of a potential carcinogen.
USP	United States Pharmacopeia

2046

X. REFERENCES

1 Development of Scientifically Justifiable Thresholds for Leachables and Extractables; PQRI Leachables and Extractables Working Group, Spring 2002. http://www.pqri.org/minutes/pdfs/dptc/lewg/workplan02.pdf

2 21 Code of Federal Regulations, Food and Drugs, Subpart A General Provisions, § 570.3, Definitions.

3 21 CFR Sec. 170.39 Threshold of regulation for substances used in food-contact articles. Federal Register: July 17, 1995 (Volume 60, Number 136, Pages 36581-96). Available electronically at: http://www.cfsan.fda.gov/~lrd/t36582.html

4 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Impurities in New Drug Substances, Q3A(R1). Available electronically at: http://www.ich.org/LOB/media/MEDIA422.pdf

5 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Impurities in New Drug Products, Q3B(R2). Available electronically at: http://www.ich.org/LOB/media/MEDIA421.pdf

6 Kroes R, Galli C, Munro I, Schilter B, Tran L-A, Walker R, Wuèrtzen G. Threshold of toxicological concern for chemical substances present in the diet: a practical tool for assessing the need for toxicity testing. Food Chem Toxicol, **38**, pp. 255-312, 2000

7 International Programme on Chemical Safety, World Health Organization (WHO). Safety evaluations of groups of related substances by the procedure for the safety evaluation of flavouring agents. In *Safety Evaluation of Certain Food Additives and Contaminants, WHO Food Additives Series*: 44. Prepared by the Fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). World Health Organization, Geneva, 2000. Accessed electronically at http://www.inchem.org/documents/jecfa/jecmono/v44jec08.htm

8 Bos PMJ, Baars B-J, van Raaij MTM. Risk assessment of peak exposure to genotoxic carcinogens: a pragmatic approach. Toxicol Lett, **151**, pp. 43-50, 2004.

9 Risk Assessment Information System, Chemical-Specific Toxicity Values. Database contains information taken from the United States Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS), the Health Effects Assessment Summary Tables (HEAST). http://risk.lsd.ornl.gov/tox/tox_values.shtml

10 California Environmental Protection Agency, Office of Environmental Health Hazard Assessment. All Chronic Reference Exposure Levels Adopted by OEHHA as of September 2002. http://www.oehha.org/air/chronic_rels/AllChrels.html

11 Rulis, A. Threshold of regulation: options for handling minimal risk situations. In Food Safety Assessment, edited by Finley, J. W., S. F. Robinson, and D. J. Armstrong. American Chemical Society Symposium Series, **484**, pp. 132-139, 1992.

12 Fiori JM, Meyerhoff RD. Extending the Threshold of Regulation Concept: De Minimis Limits for Carcinogens and Mutagens. Reg Toxicol Pharmacol, **35**, pp. 209–16. 2002

13 United States Environmental Protection Agency, Office of Water and Office of Science and Technology. Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000), October 2000, Document number EPA-822-B-00-004. Available electronically at http://www.epa.gov/waterscience/humanhealth/method/method.html

14 Reproductive and Cancer Hazard Assessment Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. Proposition 65 process for developing safe harbor numbers. February 2001. Accessed electronically at: http://www.oehha.org/prop65/policy_procedure/pdf_zip/SafeHarborProcess.pdf

15 Safety Working Party, Committee for Proprietary Medicinal Products (CPMP), the European Agency for the Evaluation of Medicinal Products (EMEA). DRAFT Position Paper on the Limit of Genotoxic Impurities. London 18 December 2002.

16 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Impurities in Residual Solvents, Q3C(R3). Appendix 4, Toxicological Data for Class 1 Solvents. Available electronically at: http://www.ich.org/LOB/media/MEDIA423.pdf

17 World Health Organization (WHO) Guidelines for drinking-water quality, 2nd ed. Vol. 2. Health criteria and other supporting information. Geneva, World Health Organization, 1996. pp. 121-131. Accessed electronically at: http://www.who.int/water_sanitation_health/GDWQ/Chemicals/Chemintro1.html#carcinogens

18 Gold LS. Carcinogenic Potency Database (CPDB). Electronic database accessed at: http://potency.berkeley.edu/cpdb.html.

19 Krewski D, Szyszkowicz M, Rosenkranz H. Quantitative factors in chemical carcinogenesis: Variation in carcinogenic potency. Regulatory Toxicol Pharmacol, **12**, pp. 13-29, 1990.

20 Pepelko WE. Effect of exposure route on potency of carcinogens. Regul Toxicol Pharmacol, **13**(1), pp. 3-17, 1991

21 EPA, 1992, "A Cross-Species Scaling Factor for Carcinogen Risk Assessment Based on Equivalence of Mg/Kg0.75/Day," Federal Register, 57: 24152-24173.

22 Physicians Desk Reference.

23 World Health Organization (WHO) Guidelines for drinking-water quality, 2nd ed. Vol. 2. Health criteria and other supporting information. Geneva, WHO, 1996. pp. 121-131. Accessed at: http://www.who.int/water_sanitation_health/GDWQ/Chemicals/Chemintro1.html#carcinogens

24 Crump K, Allen B, Ship A. Choice of dose measures for extrapolating carcinogenic risk from animals to humans: an empirical investigation of 23 chemicals. Health physics, **57**(Suppl 1), pp. 387-393, 1989.

25 Gaylor DW, Chen JJ, Sheehan DM. Uncertainty in cancer risk estimates. Risk Anal, **13**(2):149-54, 1993.

26 Fung VA, Barrett JC, Huff J. The carcinogenesis bioassay in perspective: application in identifying human cancer hazards. Environ Health Perspect, **103**(7-8), pp. 680-3, 1995.

27 Munro IC. Safety assessment procedures for indirect food additives: an overview. Reg Toxicol Pharmacol, **12**, pp. 2-12, 1990.

28 Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG, Würtzen G. Structure-based threshold of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food Chem Toxicol, **42**, pp. 65-83, 2004.

29 California Environmental Protection Agency, Office of Environmental Health Hazard Assessment. All Chronic Reference Exposure Levels Adopted by OEHHA as of September 2002. <http://www.oehha.org/air/chronic_rels/AllChrels.html>

30 Agency for Toxic Substances and Disease Registry (ATSDR). Minimal Risk Levels (MRLs) for Hazardous Substances. http://www.atsdr.cdc.gov/mrls.html

31 Alarie Y, Kane L, and Barrow CS. Sensory irritation: the use of an animal model to establish acceptable exposure to airborne chemical irritants. In "Toxicology: Principles and Practices, ed. A.L. Reeves, John Wiley, New York, pp. 48-92, 1980.

32 Kane LE, Barrow CS, and Alarie Y. A short-term test to predict acceptable levels of exposure to airborne sensory irritants. Am Ind Hyg Assoc J, **40**, pp. 207-229, 1979.

33 Schaper M. Development of a database for sensory irritants and its use in establishing occupational exposure limits. Am Ind Hyg Assoc J, **54**, pp. 488-544, 1993.

34 Cockcroft DW. Bronchial inhalation tests 1. Measurement of nonallergic bronchial responsiveness. Annals of Allergy, **55**, pp. 527-534, 1985.

35 Bohm PJA, Jorna THJM, and Henderson PT. Setting acceptable exposure limits for toluene diisocyanate on the basis of different airway effects observed in animals. Regulatory Toxicol And Pharmacol, **12**, pp. 53-63, 1989.

36 Yeates DB, Aspin N, Bryan AC, Levison H. Regional clearance of ions from the airways of the lung. Am Rev Respir Dis, **107**(4), pp. 602-608, 1973.

37 Byron PR, and Philips EM. Absorption, clearance, and dissolution in the lung. In *Respiratory Drug Delivery*, Byron PR, ed., CRC press, Boca Raton, pp 107-141, 1990.

38 Sauder LR, Green DJ, Chatham MD, Kulle TJ. Acute pulmonary response of asthmatics to 3.0 ppm formaldehyde. Toxicol Ind Health, **3**(4), pp. 569-78, 1987.

39 Avol EL, Linn WS, Shamoo DA, Anderson KR, Peng RC, Hackney JD. Respiratory responses of young asthmatic volunteers in controlled exposures to sulfuric acid aerosol. Am Rev Respir Dis, **142**(2), pp. 343-8, 1990.

40 Rubinstein I, Bigby BG, Reiss TF, Boushey HA Jr. Short-term exposure to 0.3 ppm nitrogen dioxide does not potentiate airway responsiveness to sulfur dioxide in asthmatic subjects. Am Rev Respir Dis, **141**(2), pp. 381-5, 1990.

41 Karol MH, Stadler J, and Magreni C. Immunotoxicologic evaluation of the respiratory system: animal models for immediate and delayed onset pulmonary hypersensitivity. Fundam Appl Toxicol, **5**, pp. 459-472, 1985.

42 Karol MH. Assays to evaluate pulmonary hypersensitivity. In *Methods in Immuntoxicity*, **2**, pp. 401-409, 1995.

43 O'Brien IM, Newman-Taylor AJ, Burge PS, Harries MG, Fawcett IW, Pepys J. Toluene di-isocyanate-induced asthma. II. Inhalation challenge tests and bronchial reactivity studies. Clin Allergy, **9**(1), pp. 7-15, 1979.

44 California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, All Acute Reference Exposure Levels Adopted by OEHHA as of September 2002. http://www.oehha.org/air/acute_rels/AllActRELs.html

45 Cassee FR, Groten JP, Feron VJ. Changes in the nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde, acetaldehyde and acrolein. Fund and Appl Toxicol, **29**(2), pp. 208-218, 1996.

46 Groten JP, Schoen ED, vanBladeren PJ, Kuper CF, Vanzorge JA, Feron VJ. Subacute toxicity of a mixture of nine chemicals in rats – detecting interactive effects with a fractionated two-level factorial design, Fund Appl Toxicol, **36**(1), pp. 15-29, 1997.

47 Haghdoost NR, Newman LM, Johnson EM. Multiple chemical exposure: synergism vs. individual exposure levels. Reprod Toxicol, **11**(1), pp. 9-27, 1997.

48 Jonker D, Woutersen RA, vanBladeren PJ, Til HP, Feron VJ. 4-week oral toxicity of a combination of eight chemicals in rats: comparison with the toxicity of the individual compounds. Food Chem Toxicol, **28**(9), pp. 623-631, 1990.

49 Roy, M. Age related aspects of physiology in respiratory tract modeling, in radiation protection dosimetry; Nuclear Technology Publishing, **41**(2/4), pp. 93-98, 1992.

50 Dockery, DW; Pope, CA III; Xu, X. An association between air pollution and mortality in six US cities; N Engl J Med; **329**, pp. 1753-9, 1993.

51 Daniels, MJ; Dominici, F; Samet, JM; et al. Estimating particulate matter-mortality dose response curves and threshold levels: an analysis of daily time-series for the 20 largest US cities; Am J Epidemiol, **152**(5), pp. 397-406, 2000.

52 Environmental Protection Agency, National Ambient Air Quality Standards for Particulate matter. Fed Reg, **62**, No. 138, 1997.

53 The U.S. EPA is in the process of reviewing the NAAQS for particulate matter. A draft document Review of the National Ambient Air Quality Standards for Particulate Matter: Policy Assessment of Scientific and Technical Information outlines recommendations for revision of the particulate matter standards. The draft proposes that consideration be given to the selection of the level of an annual PM2.5 standard from within the range of 15 μg/m3 to approximately 12 μg/m3 and a 24 hour PM2.5 standard from within the range of approximately 50 μg/m3 to 30 μg/m3. In addition, the draft notes the need for a new indicator, PM10-2.5. The draft explains that consideration be given to selecting an annual PM10-2.5 standard from within the range of approximately 30 μg/m3 to 13 μg/m3 and a 24 hour PM10-2.5 standard from within the range of approximately 75 μg/m3 to 30 μg/m3. There would no longer be a PM10 standard. Many aerosolized pharmaceutical products may have a particle size distribution that includes both the PM2.5 and PM10-2.5 fraction. Thus, for comparison purposes, it may be useful to consider the composite values for potential PM2.5 and PM10-2.5 standards. The composite annual values would be 45 μg/m3 to 25 μg/m3 and the composite 24 hour values would be 125 μg/m3 to 60 μg/m3.

54 Review of the National Ambient Air Quality Standards for Particulate Matter: Policy Assessment of Scientific and Technical Information, OAPQS Staff Paper, First Draft, August 2003.

55 Samet et. al. N Eng J Med, **343**, pp. 1742-1749, 2000.

56 US EPA (1985) as reported in Derelanko MJ, Holllinger MA, eds. CRC Handbook of Toxicology. Boca Raton: CRC Press, p. 643, 1995.

57 Tobin MJ, Chadha TS, Jenouri G, et al., Breathing Patterns. 1. Normal subjects. Chest, **84**(2), pp. 202-205, 1983a.

58 Tobin MJ, Chadha TS, Jenouri G, et al., Breathing Patterns. 2. Diseased subjects. Chest, **84**(3), pp. 286-294, 1983b.

59 Tobin MJ, Jenouri G, Sackner MA. Effect of naloxone on breathing patterns in patients with chronic obstructive pulmonary disease with and without hypercapnia. Respiration, **44**(6), pp. 419-424, 1983.

60 Eiguren-Fernandez A, Miguel AH, Froines JR, Thurairatnam S, Avol EL. Seasonal and spatial variation of polycyclic aromatic hydrocarbons in vapor-phase and PM2.5 in Southern California urban and rural communities. Aerosol Science and Technology, **38**, pp. 447-455, 2004.

61 From FDA blinded database of leachables in approved drug products.

62 Dourson M, Charnley G, Scheuplein R. Differential sensitivity of children and adults to chemical toxicity. II. Risk and Regulation. Regul Toxicol Pharmacol, **35**, pp. 448-467, 2002.

63 Dourson ML, Knauf LA, Swartout JC. On reference dose (RfD) and its underlying toxicity data base. Toxicol Ind Health**, 8**, pp. 171-189, 1992.

64 Burin GJ, Saunders DR. Addressing human variability in risk assessment – the robustness of the intraspecies uncertainty factor. Regul Toxicol Pharmacol, **30,** pp. 209-216, 1999.

65 Dourson M, Charnley G, Scheuplein R. Differential sensitivity of children and adults to chemical toxicity. II. Risk and Regulation. Regul Toxicol Pharmacol, **35,** pp. 448-467, 2002.

66 Bruckner JV. Differences in sensitivity of children and adults to chemical toxicity: the NAS Panel report. Regul Toxicol Pharmacol, **31,** pp. 280-285, 2000.

67 Renwick AG, Lazarus NR. Human variability and noncancer risk assessment – An analysis of the default uncertainty factor. Regul Toxicol Pharmacol, **27**, pp. 3-20, 1998.

68 Renwick AG. Toxicokinetics in infants and children in relation to the ADI and TDI. Food Addit Contam, **15,** pp. 17-35, 1998.

69 Pelekis M, Gephart LA, Lerman SE. Physiological-model-based derivation of adult and child pharmacokinetic intraspecies uncertainty factors for volatile organic compounds. Regul Toxicol Pharmacol, **33,** pp. 12-20, 2001.

70 Roy, M. Age related aspects of physiology in respiratory tract modeling, in radiation protection dosimetry. Nuclear Technology Publishing, **41**(2/4), pp. 93-98, 1992.

71 ICRP Publication 66, Human respiratory tract model for radiological protection. Ann. ICRP, **24** (1-3), 1994.

72 Musante CJ, Martonen TB. Computer simulations of particle deposition in the developing lung. J Air Waste Management Assoc, **50**, pp. 1426-1432, 2000.

73 Phalen RF, Oldham MJ. Methods for modeling particle deposition as a function of age. Respir Physiol, **128**, pp. 119-130, 2001.

74 Schiller-Scotland CF, Hlwawa R, Gebhart J, Wönne R, Heyder J. Total deposition of aerosol particles in the respiratory tract of children during spontaneous and controlled mouth breathing. J Aerosol Sci, **23**(Suppl 1), pp. S457-S460, 1992.

75 Martonen TB, Musante CJ, Segal RA, et al. Lung models: Strengths and limitations. Resp Care, **45** pp. 712-736, 2000.

76 Scheuplein R, Charnley G, Dourson M. Differential sensitivity of children and adults to chemical toxicity. I. Biological basis. Regul Toxicol Pharmacol, **35** pp. 429-447, 2002.

77 Pinkerton KE, Joad JP. The mammalian respiratory system and critical windows of exposure for children's health. Environ Health Perspect, **108**(Suppl 3), pp. 457-462, 2000.

78 Plopper CG, Hyde DM, Buckpitt AR. Clara cells. In: Crystal RG, West JB, Weibel ER, Barnes PJ (eds.). The Lung: Scientific Foundations. Second edition. Philadelphia, Lippencott – Raven, pp. 517-533, 1997.

79 Scheuplein R, Charnley G, Dourson M. Differential sensitivity of children and adults to chemical toxicity. I. Biological basis. Regul Toxicol Pharmacol, **35** pp. 429-447, 2002.

80 Barton, H, Cogliano J, Firestone, M.P., et al. Supplemental Guidance for Assessing Cancer Susceptibility from Early-Life Exposure to Carcinogens. US Environmental Protection Agency, March, 2005. EPA/630/R-03/003F http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=116283

81 Guidance for Industry, Metered Dose Inhaler(MDI) and Dry Powder Inhaler (DPI) Drug Products; Chemistry, Manufacturing, and Controls Documentation; draft Guidance; FDA, p. 13, 1998.

82 Takagi, et. al., Acute, Subchronic and Chronic Toxicity Studies of a Synthetic Antioxidant, J Toxicol Sci (Japan), **19**(2), pp 77-78, 1994.

83 Kokoski CJ, et. al. Methods Used in Safety Evaluation, in Food Additives, Chapter 15, pp 579-616, Branen AL, Davidson PM and Salminen S., eds.; Marcel Dekker, New York, 1990.

PART 3:

BEST PRACTICES FOR EXTRACTABLES AND LEACHABLES STUDIES FOR ORALLY INHALED AND NASAL DRUG PRODUCTS

I. CONTAINER/CLOSURE SYSTEM COMPONENTS – COMPOSITION AND SELECTION

A. Introduction

Selection of container/closure system components and knowledge of their composition is a vital part of extractables and leachables control in the pharmaceutical development process. Careful component selection and attention to composition information is a critical first step in the evaluation of extractables and potential leachables, as it allows the pharmaceutical development team to:

1. Obtain preliminary information on the types of potential extractables and leachables that may appear in extraction and leachables studies;

2. Develop a base of knowledge about the components which will facilitate the selection of extraction technique(s)/method(s);

3. Initiate the risk assessment process for potential extractables/leachables; and

4. Compare results of extraction studies with component compositional information as a check on the appropriateness of the extraction technique(s)/method(s).

As noted in point 3, above, component selection should include the input of toxicologists who can provide a preliminary risk assessment on compositional information provided by the supplier. Informed selection and risk assessment of components at this early stage in the development process will allow proactive assessment of compounds of potential concern, thereby saving time and resources.

The pharmaceutical development team must also identify the "critical components" of their OINDP container/closure system. The critical components of the container/closure system are defined as those that contact either the patient, i.e., the mouthpiece, or the formulation, components that affect the mechanics of the overall performance of the device, or any necessary secondary protective packaging. Pharmaceutical manufacturers and sponsors are encouraged to consult with the appropriate regulatory authorities to discuss any questions regarding the identification of critical components and their approaches to extractables and leachables evaluation and control, prior to conducting extractables and leachables studies.

B. Scope

The recommendations contained in this section address those components deemed to be "critical" for given OINDP. Description of complete selection criteria for OINDP container/closure system components is outside the scope and purpose of this document, and is not included here.

C. Recommendations for Container/Closure System Components

1. ***The pharmaceutical development team should obtain all available information on the composition and manufacturing/fabrication processes for each***

component type to the extent possible, and determine which components are "critical," before beginning extractables and leachables studies on a given OINDP and its associated container/closure system components. Such information can provide guidance as to the identities and levels of potential extractables and leachables, and includes:

(a) The elastomeric/polymeric or basic material of construction of the component, e.g., high density polyethylene, polypropylene, butyl rubber, stainless steel.

(b) The additive composition of the component, including the detailed chemical composition and reaction/degradation chemistry of each individual additive.

(c) The polymerization process, and associated polymerization/curing agents.

(d) The fabrication process, including any additives designed to assist in fabrication or processes that could result in chemical modification of any additives or the polymer, e.g., temperature.

(e) Any cleaning/washing processes for finished components, including knowledge of cleaning agents.

(f) The storage/shipping environment for both components and drug product.

Complete information should be obtained from component suppliers, to the extent practicable, on components of the OINDP container/closure system that are in contact with the formulation, the patient's mouth or nasal mucosa, or that are deemed of particular significance to the functionality of the drug product. Components in any of these three general categories are considered to be "critical components" for extractables/leachables consideration. Ancillary components including specific nebulizers and spacers that are mandated by label to be used with a specific drug product, are deemed to be critical and are therefore covered by these recommendations, and appropriate information should be obtained for these.

As an example, for an MDI (Metered Dose Inhaler) critical components would include at a minimum the canister (especially if coated), elastomeric seals, plastic valve components, metal valve components (due to surface treatments and residues) and the mouthpiece. For a DPI (Dry Powder Inhaler), critical components might include primary packaging of the individual dosage units (such as blisters, capsules, components of drug reservoirs, components of airflow pathway which may contact the drug, or films for unit dose packaging) and the DPI mouthpiece. The suppliers of OINDP container/closure systems, their components, and their principal elastomeric/polymeric or other constituents are encouraged to provide as much of the aforementioned information as possible given contractual and legal limitations. For nasal sprays and inhalation sprays,

critical components include components that are in constant contact with the formulation and components that are in the liquid pathway during actuation of the device, and that do not permit quick evaporation of residual surface liquid.

2. ***Component formulation should inform component selection.*** Early in the pharmaceutical development process, careful consideration should be given to the choice and rationale for selection of components that go into the container/closure system of the final drug product. Detailing complete selection criteria for OINDP container/closure system components is outside the scope and purpose of this document, however, it is recommended that wherever possible, the materials selected comply with accepted standards for food contact or incidental food use and/or generally recognized as safe (GRAS) materials. It is further recommended that materials used to fabricate the container/closure system meet the requirements of the indirect food additive regulations in Title 21 of the Code of Federal Regulations, where applicable.[1] In addition, certain specified materials used to fabricate the components of the container/closure system should be tested according to USP <87> and <88>. (This applies to MDI components that contact the drug formulation and the patient; to the DPI mouthpiece; and nasal spray and inhalation solution, suspension and spray container, closure, and critical pump components).

Components containing sources of known potent carcinogens or mutagens should be avoided or minimized, e.g., Polynuclear Aromatic Hydrocarbons [PAHs or PNAs] in carbon black filler, N-nitrosamines in various sulfur-cured elastomers, or mercaptobenzothiozole in certain elastomer sulfur curing agents. It is recommended that the manufacturing/fabrication processes for elastomeric and plastic components be optimized so as to minimize the requirement for, and levels of, chemical additives and/or processing aids. It is further recommended that where possible, elastomeric components be subject to washing or other cleaning processes designed to remove/minimize extractables. Such cleaning processes should be validated for this purpose, and should in no way compromise the functionality of the component. A desirable goal is to have component manufacturing/fabrication processes under cGMP (current Good Manufacturing Practices) control, with associated in-process controls and quality assurance auditing practices.

Note, however, that the selection process and information from suppliers does not preclude the need for conducting comprehensive Controlled Extraction Studies and appropriate safety qualification of leachables.

3. ***Risk Assessment should be performed during the selection of components and materials.*** As part of the process for selecting materials and components for OINDP, the sponsor should conduct risk assessment on the component based on information from the supplier regarding the identity and amounts of ingredients in a component or material. Given this information the pharmaceutical development team toxicologist(s) should estimate worst-case total daily intake (TDI) for ingredient compounds. If available, chemical structures of additives and other

ingredients should be provided to the toxicologist, allowing conduct of SAR studies and literature searches to provide an estimate of potential risk if these compounds were to appear in a drug product leachables profile. Based on this risk assessment, the sponsor may choose to select different components/materials or discuss with the supplier how the concentration of an ingredient in a component/material might be decreased.

4. ***Extractables testing, including Controlled Extraction Studies and the development and validation of Routine extractables testing methods, should be accomplished for all critical OINDP components.*** Appropriate characterization and control of extractables profiles in non-patient-contact critical components should also be accomplished. Recommendations for the design and conduct of extractables testing are detailed in the following chapters of this recommendation document.

D. Examples Illustrating Recommendations 1 and 3: Knowledge Derived from Component Composition and Risk Assessment

1. Recommendation – Obtain Composition Information from Suppliers

The Working Group obtained both plastic and elastomeric test articles, as these types of materials are typically used in a wide variety of OINDP. Specifically, the test articles were polypropylene, a sulfur-cured elastomer, and a peroxide-cured elastomer. All of these test articles were manufactured specifically for the Working Group so that their full composition could be divulged without the need for formal contractual obligations. A fourth test article (a second peroxide cured elastomer) was also obtained, but the formulation for this material was purposefully not provided to the Group until after extraction studies were performed on the material, in order to further investigate the need for thorough extraction studies. Note that the compositions of the test articles in these studies do not necessarily correspond to the proprietary formulations used in OINDP components.

Two questions are central to this study, and guided the Working Group in obtaining and then evaluating the formulation information:

- What kind of knowledge can a pharmaceutical development team derive from information about OINDP container/closure system critical components provided by suppliers?

- How is such knowledge useful in the design of Controlled Extraction Studies, Leachables Studies, and development/validation of Routine Extractables Testing methods for critical components?

As an example, consider the available information on the compositions of test articles used in the PQRI Leachables and Extractables Working Group's laboratory Controlled Extraction Studies.

The compositions, as provided by the suppliers, of the sulfur-cured and polypropylene test articles are shown below in Tables 1 and 2:

2223

Table 1. Ingredients In Sulfur-Cured Elastomer Test Article

Ingredient	Registry #(S)	Percent (w/w)
Calcined Clay	308063-94-7	8.96
Blanc Fixe (Barium Sulfate)	7727-43-7	25.80
Crepe	9006-04-6	38.22
Brown Sub MB (Ingredients Below)	NA (not available)	16.84
Brown Sub Loose	NA	33.30
Crepe	9006-04-6	66.70
1722 MB (Ingredients Below)	NA	2.11
SMR (Standard Malaysian Rubber)	NA	60.00
FEF Carbon Black (Low PNA)	1333-86-4	40.00
Zinc Oxide	1314-13-2	4.04
2, 2' Methylene-bis (6-*tert*-butyl-4-ethyl phenol)	88-24-4	0.56
Coumarone-Indene Resin	164325-24-0 140413-58-7 140413-55-4 68956-53-6 68955-30-6	1.12
Paraffin	8002-74-2 308069-08-1	1.12
Tetramethylthiuram Monosulfide	97-74-5	0.11
Zinc 2-Mercaptobenzothiazole	149-30-4 155-04-4	0.29
Sulfur	7704-34-9	0.84

2224

Table 2. Ingredients in Polypropylene Test Article

Ingredient			Percent (w/w)
Chemical Name	**Registry #**	**Commercial Name**	
Tetrakis (methylene(3,5-di-*tert*-butyl-4-hydroxyhydrocinnamate)) methane	6683-19-8	Irganox 1010 Anox 20	0.08
Bis(2,4-di-*tert*-butylphenyl)pentaerythritol diphosphite	26741-53-7	Ultranox 626	0.05
Calcium Stearate	1592-23-0	NA	0.03 - 0.4
Vegetable oil derived 90% alpha	31566-31-1	Pationic 901	0.3

monoglycerides		Dimodan HS-KA	
3,4 -dimethyldibenzylidene sorbitol	135861-56-2	Millad 3988	0.2

The compositional information detailed in Tables 1 and 2 provides the following knowledge, useful in the design of extractables/leachables studies:

(i) Sulfur-cured Elastomer

a. The presence of carbon black implies the possible presence of PNAs, which might appear as extractables/leachables. Since PNAs are a compound class of special concern, this knowledge would initiate special analytical investigations, which would require specific and highly sensitive analytical techniques and methods.

b. Sulfur curing agents, for example tetramethylthiuram monosulfide, suggest the potential presence of N-nitrosamines, which might appear as extractables/leachables. As with PNAs, N-nitrosamines are a compound class of special concern and this knowledge would initiate special analytical investigations, again with specific and highly sensitive analytical techniques and methods.

Note: ***The presence of PNAs and N-nitrosamines should be assessed in the development process, regardless of the composition of the elastomeric component.***

c. The presence of 2-mercaptobenzothiazole, another special case compound, would also initiate special analytical investigations.

Note: ***Armed with information regarding PNAs, N-nitrosamines, and 2-mercaptobenzothiazole as potential extractables/leachables, a pharmaceutical development team might want to reassess the use of components manufactured with this particular elastomer in the proposed OINDP container/closure system.***

d. Paraffin and Coumarone-indene resin are natural product materials, and are therefore likely to produce complex extractables/leachables profiles containing many related chemical entities.

e. The individual additives 2,2'-methylene-bis(6-*tert*-butyl-4-ethylphenol), tetramethylthiuram monosulfide, 2-mercaptobenzothiazole, and sulfur will likely be analyzable by Gas Chromatographic (GC) techniques; however, due to the complex nature of the elastomer formulation, use of High-Performance Liquid Chromatography (HPLC) techniques is also advisable in order to provide a more complete representation of the extractables/leachables profiles. Further, because such additives are potentially complex and can have potentially complex reaction/degradation chemistries, it may be desirable to obtain these individual additives so as to understand their compositions, chemistries and analytical properties. The lack of trade names for individual additives complicates this process.

(ii) Polypropylene

a. Polypropylene is known to generate potentially significant numbers and levels of soluble oligomers (for instance, soluble in CFC propellants of MDI formulations), which might appear as both extractables and leachables. Since such soluble oligomers are known to be relatively volatile and non-polar, Gas Chromatographic (GC) analysis of both extractables and leachables is indicated.

b. The chemical properties, e.g., molecular weight, volatility, potential degradation chemistry, of additives such as Irganox 1010, Ultranox 626, and Millad 3988 suggest that GC analysis alone will be insufficient to adequately characterize extractables and leachables from these chemical substances. The use of HPLC based analytical methods is therefore indicated.

c. Additives such as Pationic 901 are potentially chemically complex, and individual additives Irganox 1010, Ultranox 626, and Millad 3988 could have complex degradation chemistries. Therefore, it may be desirable to obtain these individual additives so as to understand their compositions, chemistries and analytical properties. The availability of trade names facilitates this.

d. There is no reason to suspect the presence of special case compounds or compound classes, e.g., PNAs, N-nitrosamines, 2-mercaptobenzothiazole, in this test article, and therefore special analytical studies designed to characterize these chemical entities are not required.

As shown in the above examples, significant information can be obtained from information available from OINDP component suppliers. Many of the individual ingredients have one or more registry numbers, which allows for computerized database reference searching. Example searches using registry numbers for Irganox 1010 and 2-mercaptobenzothiazole yielded 4709 and 6343 citations respectively. This information should facilitate the selection of components for use in OINDP container closure systems, and the design of extractables/leachables studies for OINDP pharmaceutical development programs.

However, such information, no matter how detailed, does not preclude the need for completing comprehensive Controlled Extraction Studies and Leachables Studies, followed by the development and validation of Routine Extractables Testing analytical methods for extractables, for critical components fabricated from these materials.

2. **Recommendation – Conduct Risk Assessment Based on Supplier Information**

Risk assessment using information from suppliers can be performed by calculating a estimated worst-case Total Daily Intake (TDI) from the data provided. An example of how TDIs for risk assessment can be estimated from supplier information is presented below using the ingredients list for the sulfur-cured elastomer in Table 1, and a hypothetical drug product configuration and amount of material.

Given a 200 dose product, 150 mg of elastomer with compound 2, 2’ methylene-bis (6-

tert-butyl-4-ethyl phenol) at 0.56 percent w/w,

(0.0056) × (150 mg/elastomer) ÷ (200 doses/product) = 0.0042 mg/dose = 4.2 µg/dose

If the product configuration requires 4 doses/day then,

(4.2 µg/dose) × (4 doses/day) = 16.8 µg/day

Thus the estimated worst-case TDI is 16.8 µg/day. Given this estimated TDI, SAR assessments on the compound, and literature searches on the safety implications of the compound, the sponsor can determine the risk involved in using this material.

E. References

1 Code of Federal Regulations, Title 21, Parts 174-178; http://www.access.gpo.gov/nara/cfr/waisidx_04/21cfrv3_04.html

II. CONTROLLED EXTRACTION STUDIES

A. Introduction

After a thorough evaluation of the available information on component formulation and fabrication processes, an OINDP pharmaceutical development team should begin the extractables and leachables testing process by conducting Controlled Extraction Studies on all critical components of the OINDP container/closure system. The significance and impact of properly conducted and evaluated Controlled Extraction Studies on the OINDP pharmaceutical development process cannot be overstated.

A Controlled Extraction Study is a laboratory investigation into the qualitative and quantitative nature of extractables profiles of critical components of an OINDP container/closure system. The purpose of a Controlled Extraction Study is to systematically and rationally identify and quantify potential leachables, i.e., extractables, to the extent practicable, and within certain defined analytical threshold parameters. Controlled Extraction Studies typically involve vigorous extractions of representative lots of components using multiple solvents of varying polarity, with both qualitative and quantitative evaluation of the resulting extractables profiles. Multiple analytical techniques/methods with compound specific detection, e.g., mass spectrometry, are usually employed to establish extractables profiles. It is often the case that the analytical techniques/methods used in Controlled Extraction Studies, along with the qualitative and quantitative results of these studies are used to:

1. Establish a basis for the development and validation of routine quality control methods and acceptance criteria for critical component extractables profiles.

2. Establish a basis for the development and validation of leachables methods suitable for use in drug product leachables studies as well as for potential use as routine quality control methods for drug product leachables (should such be required by regulatory authorities).

3. Allow for the "correlation" of extractables and leachables.

The Controlled Extraction Study can be framed as a problem in the general field of Trace Organic Analysis (TOA).[1,2] In a TOA problem, a complex mixture of trace level organic chemical entities, i.e., extractables, contained within a matrix, e.g., rubber, plastic, is recovered from the matrix, i.e., extracted, and the individual organic chemical entities are identified and/or quantified. Jenke[3] has provided a comprehensive discussion and classification of extraction strategies that can be used for Controlled Extraction Studies, intended ultimately for drug product leachables assessments. He states two so-called "directives" with which all Controlled Extraction Study extraction techniques/methods must comply. For OINDP Controlled Extraction Studies these may be restated as follows:

1. Extraction techniques/methods used for Controlled Extraction Studies should be vigorous, but not so aggressive as to alter the qualitative and/or quantitative nature of the extractables profile, and therefore preclude an extractables/leachables correlation.

2. Extraction techniques/methods used for Controlled Extraction Studies must be technically justified and optimized to produce extractables profiles at least equivalent to leachables profiles obtained under worst case conditions of drug product use, allowing both qualitative and quantitative extractables/leachables correlations.

Properly conducted Controlled Extraction Studies, when accomplished early in the pharmaceutical development process, permit a pharmaceutical development team to begin early evaluation of potential drug product leachables. This evaluation can alert the pharmaceutical development team to potential leachables with toxicological concerns, allowing adequate time to begin appropriate safety qualification studies, if necessary, or modification of the container/closure system component(s). Toxicology studies are time-consuming and modifications to container/closure system components are most easily made early in the pharmaceutical development process. Early and well-designed Controlled Extraction Studies are therefore critical to reducing the time and cost of an OINDP pharmaceutical development program.

The PQRI Leachables and Extractables Working Group conducted Controlled Extraction Studies on specially created rubber and plastic test articles (see Chapter I, Component Selection). Based on the results of these studies, and the knowledge and experiences of Working Group members, "best practice" recommendations for the conduct of Controlled Extraction Studies were developed and proposed. These recommendations are summarized and subsequently described in detail below. Data from the Working Group's Controlled Extraction Studies are used in support of individual recommendations.

B. Scope and Application for Controlled Extraction Studies

Controlled Extraction Studies should be accomplished on all critical components incorporated into the container/closure systems of every type of OINDP (see ***I. Component Selection***, for discussion of critical components). For Metered Dose Inhalers (MDIs), Controlled Extraction Studies must be accomplished on all dose metering valve elastomeric and plastic components, the inner surface of the metal canister (should the canister be coated), and the actuator/mouthpiece. Note that for uncoated metal canisters and certain metallic valve components it is necessary to accomplish surface extraction studies to identify and quantify any oily processing residues which may be present.

For Dry Powder Inhalers (DPIs), Controlled Extraction Studies must be accomplished on all elastomeric and plastic components which are in direct contact with either the patient's mouth or nasal mucosa, and/or in contact with the drug product or dry product stream. This is not limited to the DPI itself, but should also include the container/closure system for the drug product unit doses, e.g., plastic or foil blisters, laminates. Any glues or other adhesives involved must also be considered. Since consideration of non-contact critical components is of particular concern for DPIs, other non-contact components which are critical to the performance of the DPI system may still require Controlled Extraction Studies as a prelude to the development and validation of routine quality control methods for extractables profiles. Given that the extractables profile is an indicator of chemical additive composition of the component, and the additive composition is a potential indicator of physical performance of the component,

extractables profile controls on non-contact critical components may be of benefit to drug product quality. DPI pharmaceutical development teams are encouraged to consult the regulatory authorities regarding the identification of critical components early in the development process (see Chapter I, Component Selection).

For Inhalation Solutions and Spray drug products, Controlled Extraction Studies should be accomplished for any components with drug product or patient contact (plastic containers, plastic bottles, dip tubes, etc.). Migration of potential leachables through semi-permeable plastic containers (fabricated from low density polyethylene, for example) is of particular concern for inhalation solution, suspension and spray products. Sources of migrants include labels, inks, adhesives, etc., in direct contact with the outer surface of the plastic container, and volatiles from external sources not in direct contact. External sources can include cardboard shipping containers, plastic coatings on the inner surface of a foil overwrap, etc. OINDP pharmaceutical development teams should carefully consider possible sources of potential leachable migration and conduct appropriate Controlled Extraction Studies in order to identify and quantify these potential leachables.

For all OINDP critical components, it is important to remember that component fabrication and processing can potentially add extractables, i.e., potential leachables, in addition to what is expected from the known component formulation. These could include mould release agents, antislip agents, antistatic agents, lubricants, and others.

C. Recommendations for Controlled Extraction Studies

1. ***Controlled Extraction Studies should employ vigorous extraction with multiple solvents of varying polarity.*** The function of the critical component along with knowledge of component composition and drug product formulation should be used to guide solvent selection. For example, methylene chloride (or dichloromethane) is a good solvent to use for MDI valve components, since it is reasonable to assume that it will have similar extracting properties to typically used MDI propellants. It is reasonable (and essential) to use water for Controlled Extraction Studies of Inhalation Solution critical components where the drug product formulation is aqueous based. However, water should not be the only extracting solvent used for components from aqueous based drug products, and would never be an appropriate choice for an MDI valve component when the MDI propellant is either CFC or HFA based. While knowledge of component composition is a useful guide, one should never assume that such knowledge can be used to completely define an extractables profile. Solvents with a range of polarities, e.g., methylene chloride, isopropanol, hexane, should be selected to cover a wide range of potential extractables. The solvents selected should maximize both the number of extractable compounds and their levels, within the directives of Jenke as discussed above. The preceding statement, and above recommendation that extractions should be “vigorous”, is not meant to imply that 100% of the known additives should be extracted from critical component materials. Such extractions, often termed “deformulation”, are likely in many cases to produce extractables profiles which violate Jenke’s criteria and are difficult to correlate with drug product leachables profiles. It should be

remembered that certain solvents are potentially reactive, e.g., methanol, ethanol, or contain potentially reactive contaminants, e.g., ethyl ether, tetrahydrofuran, and their use in Controlled Extraction Studies should be justified. In addition, results should be carefully evaluated with respect to extraction artifacts. Extraction artifacts are peaks not related to extractables, but which may be generated by the analytical method used. In general, extractables profiles should be carefully evaluated for extraction artifacts.

2. ***Controlled Extraction Studies should incorporate multiple extraction techniques.*** Extraction techniques can be complementary. For example, methylene chloride sonication and methylene chloride reflux are performed at different temperatures, and extraction kinetics are obviously temperature dependent. The use of multiple extraction techniques along with multiple solvents allows for a more informed decision when choosing an extraction process to optimize for extractables/leachables correlation, development/validation of routine extractables control methods, etc. Examples of extraction technique choices include, but are not limited to, Soxhlet, reflux, and sonication. This recommendation does not preclude the use of automated or instrument based extraction techniques, such as Accelerated Solvent Extraction (ASE), Super-critical Fluid Extraction (SFE), or microwave extraction. The Working Group recognizes that in certain specific situations such as migration of chemical entities through the gas phase in a DPI unit dose blister, Controlled Extraction Studies that do not use a solvent are appropriate. Such studies may be collectively refered to as "volatile studies," and often require special instrumentation and equipment. These Recommendations do not preclude the accomplishment of volatile studies, as appropriate. The Working Group does not intend to recommend, endorse, or preclude any particular extraction technique or process as there are a number of equally acceptable choices for any particular critical component application, however, the pharmaceutical development team should be aware that beakers, flasks and other glassware will likely be available many years and decades into the future while particular instruments might not be. As stated above, extraction temperature can be a factor affecting both extraction efficiency and the formation of extraction artifacts. Low temperature extraction techniques such as sonication, should be justified regarding their extraction efficiency, while extractables profiles from higher temperature extraction techniques should be carefully examined for extraction artifacts.

3. ***Controlled Extraction Studies should include careful sample preparation based on knowledge of analytical techniques to be used.*** When using Gas Chromatography (GC) based analytical techniques, it is not always appropriate to inject high-boiling or reactive solvents, therefore it might be necessary to switch solvents prior to extractables profile analysis. For example, it is usually inappropriate to inject water extracts directly into a GC, so it is necessary to extract the organic compounds out of the water sample with a more non-polar solvent prior to GC analysis. Likewise, when using Liquid Chromatography (LC) based analytical techniques it is usually inappropriate to inject samples in solvents which are not miscible in the mobile phase. For example, methylene chloride

extracts should probably be dried or significantly concentrated (if taking to complete dryness is demonstrated to be a problem) and the extractables redissolved in mobile phase prior to analysis. For any sample preparation strategy, the implications for recovery and/or loss of extractables should be considered.

4. ***Controlled Extraction Studies should employ multiple analytical techniques.*** No single analytical technique will be sufficient to detect and/or identify all possible extractables from any particular container/closure system component, therefore, multiple broad spectrum techniques should be used to ensure complete evaluation of an extractables profile. For identification of individual extractables, analytical techniques should have "compound specific" detection. That is, the detector should provide information unique to the molecular structure of an individual chemical entity. Further, the detector's response should in some way be proportional to the amount of each individual extractable so that extractables profiles are quantitative. Commonly used analytical techniques for Controlled Extraction Studies involve the combination of chromatography with mass spectrometry, for instance Gas Chromatography/Mass Spectrometry, GC/MS; Liquid Chromatography/Mass Spectrometry, LC/MS. Other analytical techniques, such as liquid chromatography with photodiode array detection (LC/DAD) can also be employed.

5. ***Controlled Extraction Studies should include a defined and systematic process for identification of individual extractables.*** It is vital that the data and processes used to identify, i.e., elucidate the chemical structure of, individual extractables be clearly defined and understood. Given the large number of potential extractables, it is not reasonable to expect that authentic reference compounds will be available to confirm every identification. Therefore, other levels of identification confidence must be employed and evaluated by regulatory authorities. Note that at the level of the Qualificiation Threshold (QT), complete identification of an extractable or leachable should be possible.

6. ***Controlled Extraction Study "definitive" extraction techniques/methods should be optimized.*** After evaluating extractables profiles from various extraction techniques/methods and solvents, a pharmaceutical development team should choose a "definitive" extraction technique(s)/method(s) to optimize. An optimized extraction method is defined as one that yields a high number and concentration of extractables, and achieves steady-state levels, i.e., "asymptotic levels," without violating Jenke's directives discussed previously. Optimization of the extraction technique(s)/method(s) prior to conducting quantitative Controlled Extraction Studies ensures that the extractables profile(s) represents at least a "worst-case" scenario of potential leachables and their levels. Extractables profiles produced from such optimized technique(s)/method(s) should be thoroughly evaluated both qualitatively and quantitatively (see Chapter IV, The AET, for discussion of quantitative evaluation). While complete validation is not recommended or expected for Controlled Extraction Study methods, it is recommended that appropriate experiments be accomplished to verify that

quantitative results are accurate and precise. This is especially true if the quantitative Controlled Extraction Study results are an integral part of a quantitative extractables/leachables correlation. Appropriate method verification experiments could include evaluations of precision, accuracy, linearity, selectivity, etc.

7. ***During the Controlled Extraction Study process, sponsors should revisit supplier information describing component formulation.*** The sponsor should develop a comprehensive identified list of extractables that could be potential leachables, and should check this list against available supplier information. The sponsor should compare results of the Controlled Extraction Studies, e.g., identity and amount of extractables, with the supplier information to determine if the extraction and analysis methods used are appropriate, and to determine the presence of other chemical entities not included in the supplier information. Alternatively, the sponsor/applicant can use supplier information about the composition of materials as a starting point for the development of appropriate qualitative and quantitative methods, which may then be used to analyze the extractables obtained. The extractable profile may then be compared with supplier information (see Chapter I, Component Selection, for details on supplier information).

8. ***Controlled Extraction Studies should be guided by an Analytical Evaluation Threshold (AET) that is based on an accepted safety concern threshold.*** The AET is designed to establish how low one should go in a given extractables profile to identify and evaluate individual extractables. A complete discussion of the AET is presented in Part 3, Chapter IV of this recommendation document.

9. ***Polycyclic Aromatic Hydrocarbons (PAH's; or Polynuclear Aromatics, PNA's), N-nitrosamines, and 2-mercaptobenzothiazole (MBT) are considered to be "special case" compounds, requiring evaluation by specific analytical techniques and technology defined thresholds.*** These particular compound classes and chemical entities have historically demanded greater scrutiny and are therefore considered separately from other extractables.

10. ***Qualitative and quantitative extractables profiles should be discussed with and reviewed by pharmaceutical development team toxicologists so that any potential safety concerns regarding individual extractables, i.e., potential leachables, are identified early in the pharmaceutical development process.*** Early safety review of extractables profiles obtained during Controlled Extraction Studies has significant potential benefit to the pharmaceutical development process for OINDP. Potential leachables which represent possible safety concerns can be identified and evaluated at a point in the process where corrective changes to the container/closure system would have less effect on the timeliness and cost of the OINDP development program. Therefore, the results of Controlled Extraction Studies should also be used as a component and material selection tool.

D. Discussion and Supporting Data for Recommendations

This section presents more detailed discussion and supporting data for each of the recommendations listed in Section II.C. The data were acquired during the Controlled Extraction Studies performed on custom made elastomer and plastic test articles by the volunteer laboratories of the Working Group. These studies were intended to represent those studies that might be employed for MDI valve critical components, and were conducted according to protocols, reproduced in Appendix 4 and developed by the Working Group. The interested reader is referred to these protocols for experimental details. The Controlled Extraction Studies were both qualitative and quantitative, and example data from both studies are presented in this chapter and other chapters in the recommendation document.

To summarize:

- Extractables profiles were obtained from four custom made test articles (one sulfur-cured elastomer, 2 peroxide-cured elastomers, one polypropylene).
- Each test article was extracted by three extraction techniques (Soxhlet, reflux, sonication).
- Each test article and extraction technique employed three solvents (methylene chloride, 2-propanol, hexane).
- Extracts were analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Mass Spectrometry (LC/MS), and Liquid Chromatography/Ultraviolet detection (LC/UV) generating extractables profiles.
- For three of the test articles (sulfur-cured rubber, one peroxide-cured rubber, and polypropylene) an extraction technique/solvent system was chosen and optimized.
- Extractables were identified using a systematic process with defined identification criteria.
- Quantitative Controlled Extraction Studies were accomplished for two of the test articles (sulfur-cured rubber and polypropylene) with the optimized extraction techniques/methods and solvent systems.
- For the sulfur-cured rubber, a “special case” compound (2-mercaptobenzothiazole) was investigated with a specific analytical technique/method.

1. Recommendation - Use of Multiple Solvents

The Working Group chose the following solvents for use in its Controlled Extraction studies on the custom made elastomeric and plastic test articles:

- methylene chloride (dichloromethane)
- 2-propanol (isopropanol)

- hexane (n-hexane)

These solvents were chosen because:

1. They represent a range of polarities, and therefore potential solubilizing properties.
2. They represent a range of boiling points.
3. They are relatively non-reactive chemically.
4. They are easily and safely handled in a typical analytical laboratory setting.
5. They are readily available in high purity.

Note that one of the solvents used during Controlled Extraction Studies should have similar extracting properties to the drug product vehicle. Since the Working Group's Controlled Extraction Study was intended as an MDI critical component study, methylene chloride was chosen to mimic CFC and HFA propellants and isopropanol was chosen to mimic ethanol (a common cosolvent for MDI drug product formulations). In the case of Inhalation Solutions and other aqueous based drug products, water or another aqueous based medium, e.g., aqueous buffer solution, should be used as an extracting solvent. In certain cases it may be possible to use the actual drug product vehicle as an extracting medium for Controlled Extraction Studies, and this is encouraged by the Working Group.

The Controlled Extraction Study results provide many examples of the utility of using multiple solvents of varying chemical and physical properties. Figures 1 and 2 show HPLC/DAD (High Performance Liquid Chromatography/Diode Array Detection) extractables profiles, i.e., chromatograms, of 2-propanol and hexane extracts of the polypropylene test article. Note that the polypropylene under hexane reflux (Figure 1) yields tetrakis[methylene (3,5-di-*tert*-butyl-4-hydroxyhydrocinnamate)] methane (Irganox 1010) and bis(2,4-di-*tert*-butylphenyl)pentaerythritol diphosphite (Ultranox 626). However, the presence of 3,4-dimethyl dibenzylidene sorbitol (Millad 3988) was only confirmed via results obtained from the 2-propanol reflux (Figure 2). Since Millad 3988 is a known primary ingredient in the polypropylene formulation, the extracting/solubilizing power of the 2-propanol is of clear utility.

Note that the small peak at approximately 2.5-3 minutes in Figure 1 is not Millad 3988. This was confirmed by retention time and UV spectral match. In Figure 2, the peak at approximately 2 minutes is the peak for the 2-propanol solvent.

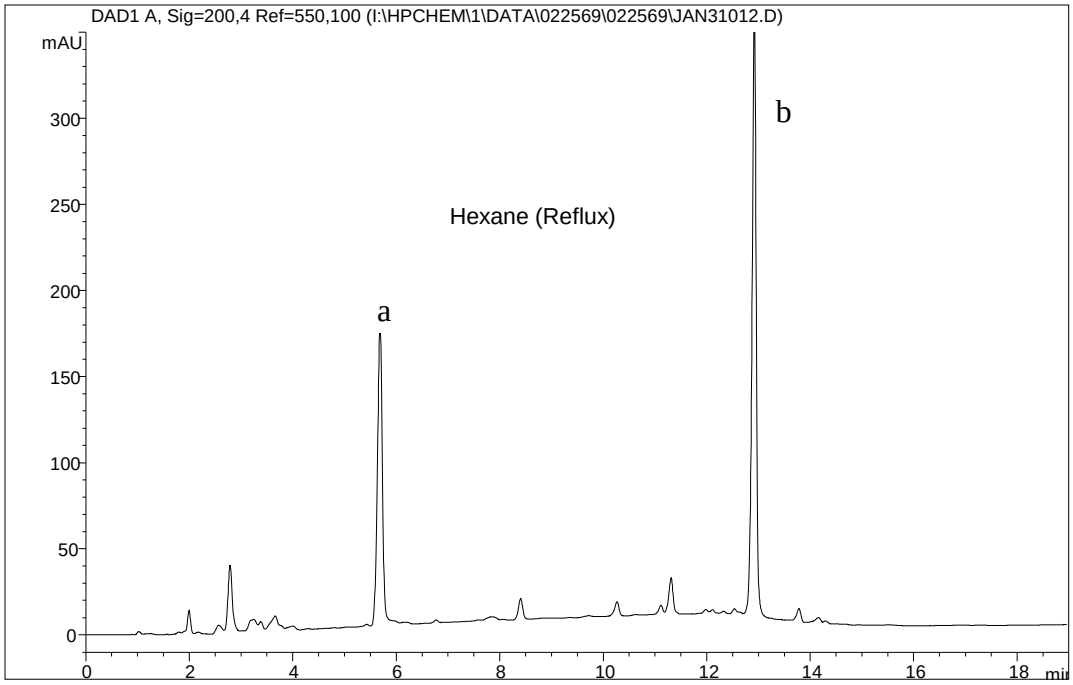


Figure 1. HPLC/DAD (High Performance Liquid Chromatography/Diode Array Detection) extractables profile (UV@200 nm) of a polypropylene test article hexane reflux extract. **a** = di-*tert*-butylphenol from Ultranox 626; **b** = Tetrakis [methylene (3,5-di-*tert*-butyl-4-hydroxyhydrocinnamate)] methane (Irganox 1010).

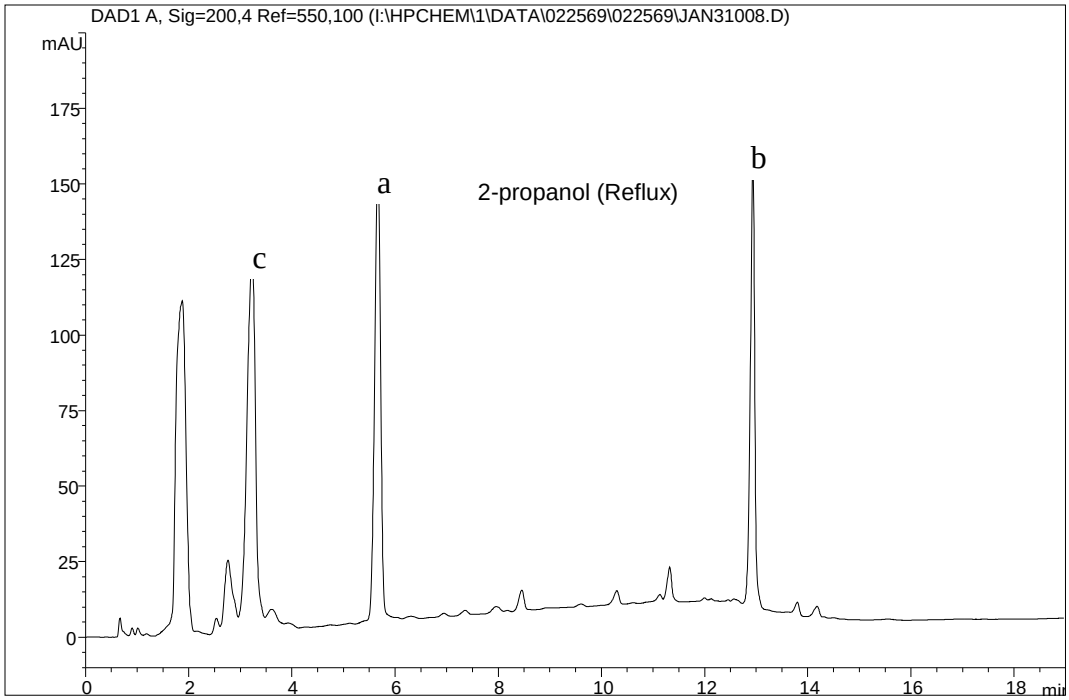
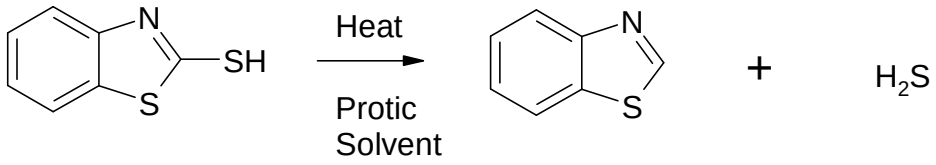


Figure 2. HPLC/DAD (High Performance Liquid Chromatography/Diode Array Detection) extractables profile (UV@200 nm) of a polypropylene test article 2-propanol reflux extract. a = di-*tert*-butylphenol from Ultranox 626; b = Tetrakis [methylene (3,5-di-*tert*-butyl-4-hydroxyhydrocinnamate)] methane; c = 3,4-dimethyl dibenzylidene sorbitol. The peak at approximately 2 minutes represents 2-propanol.

Figures 3, 4 and 5, show extractables profiles in the form of GC/MS Total Ion Chromatograms (TICs) from 2-propanol, hexane and methylene chloride reflux extracts of the sulfur-cured elastomer. Note that the profiles differ in number and intensity of peaks depending on the solvent used, a significant observation which favors the use of multiple solvents. The major peak in all three extractables profiles was confirmed to be the phenolic antioxidant 2,2'-methylene-bis-(6-*tert*-butyl)-4-ethylphenol, a known elastomer formulation ingredient. Of particular note in Figure 4, however, is the peak at approximately 8 minutes retention time which is not so apparent in Figures 3 and 5. This extractable was identified as benzothiazole (**II**), and its presence in the 2-propanol reflux extract at this relatively high level is likely the result of thermolysis of the known ingredient 2-mercaptobenzothiazole (**I**). The boiling points of the extracting solvents are, respectively: methylene chloride 40.1°C, 2-propanol 82.3°C, and n-hexane 69.0°C. It is attractive to hypothesize that the higher temperature at which the protic solvent 2-propanol is refluxing is responsible for the high level of benzothiazole, as follows:



I **II**

At first reading this would appear to be an extraction artifact, however it is important to point out that comparison of these three extractables profiles along with a basic understanding of organic chemistry and chemical reactivity, would alert the analytical chemist to the potential presence of the special case extractable 2-mercaptobenzothiazole.

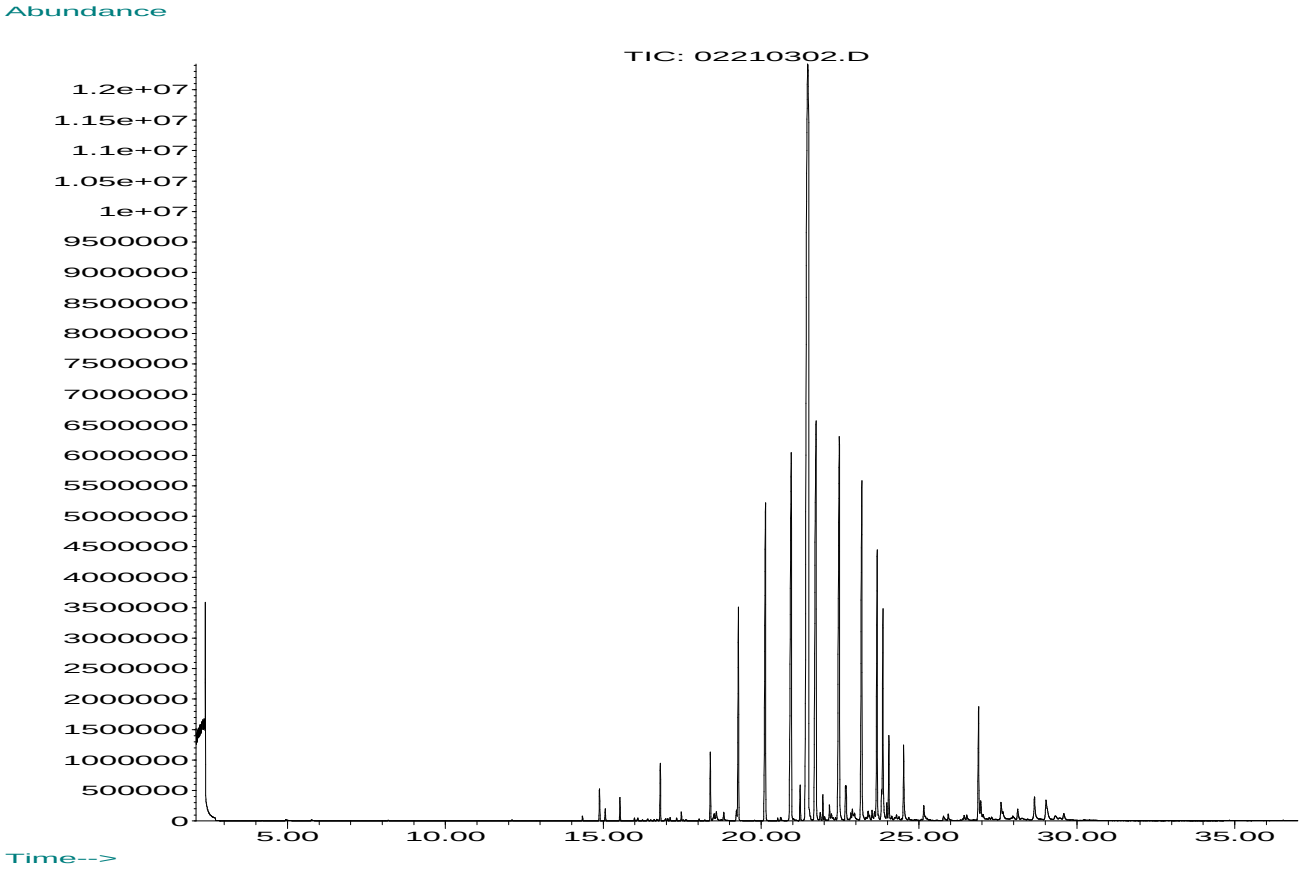


Figure 3. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the sulfur-cured elastomer test article, methylene chloride reflux extract.

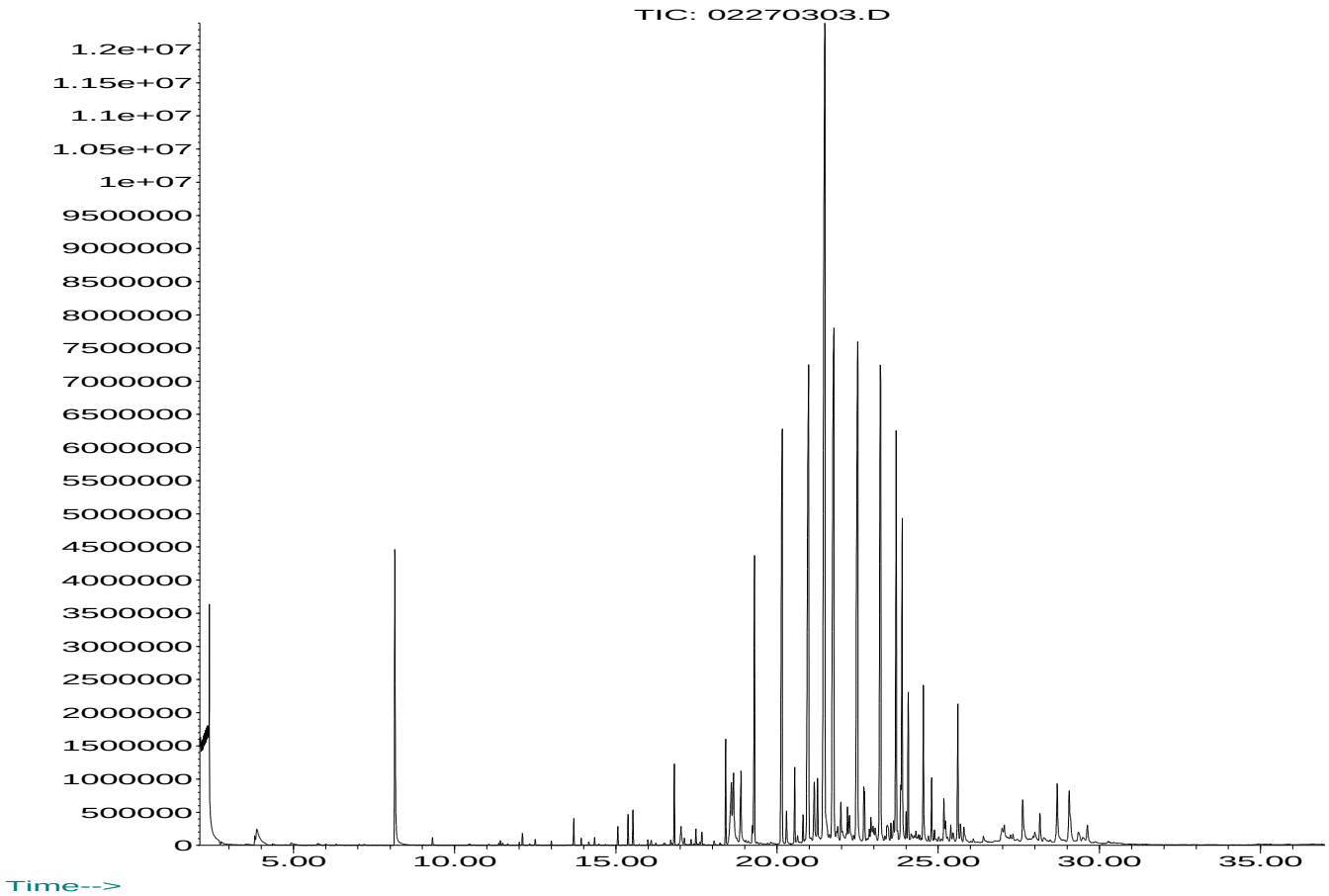


Figure 4. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the sulfur-cured elastomer test article, 2-propanol reflux extract. Sample reconstituted in methylene chloride prior to GC/MS analysis.

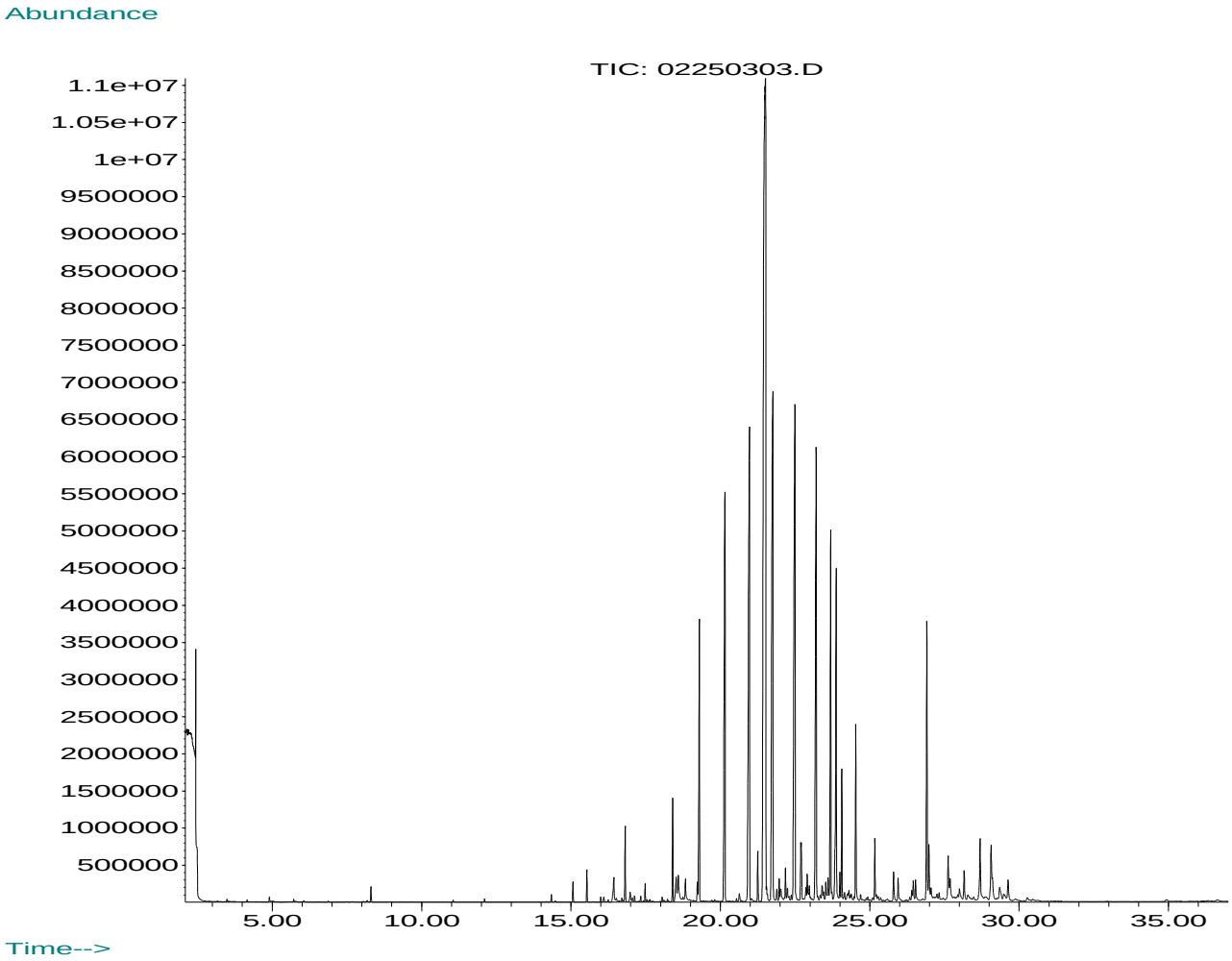


Figure 5. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the sulfur-cured elastomer test article, hexane reflux extract.

Figures 6, 7, and 8 show GC/MS TICs of extracts from one of the peroxide-cured elastomers after Soxhlet extraction in methylene chloride, 2-propanol, and hexane. Again, profiles differ depending on the solvent used. Qualitatively, methylene chloride appears to provide the best yield of different types of potential extractables. For instance, the suite of peaks from about 5 to 15 minutes retention time is quite prominent in the methylene chloride extractables profile. These peaks are moderately apparent in the hexane study and not apparent in the 2-propanol results.

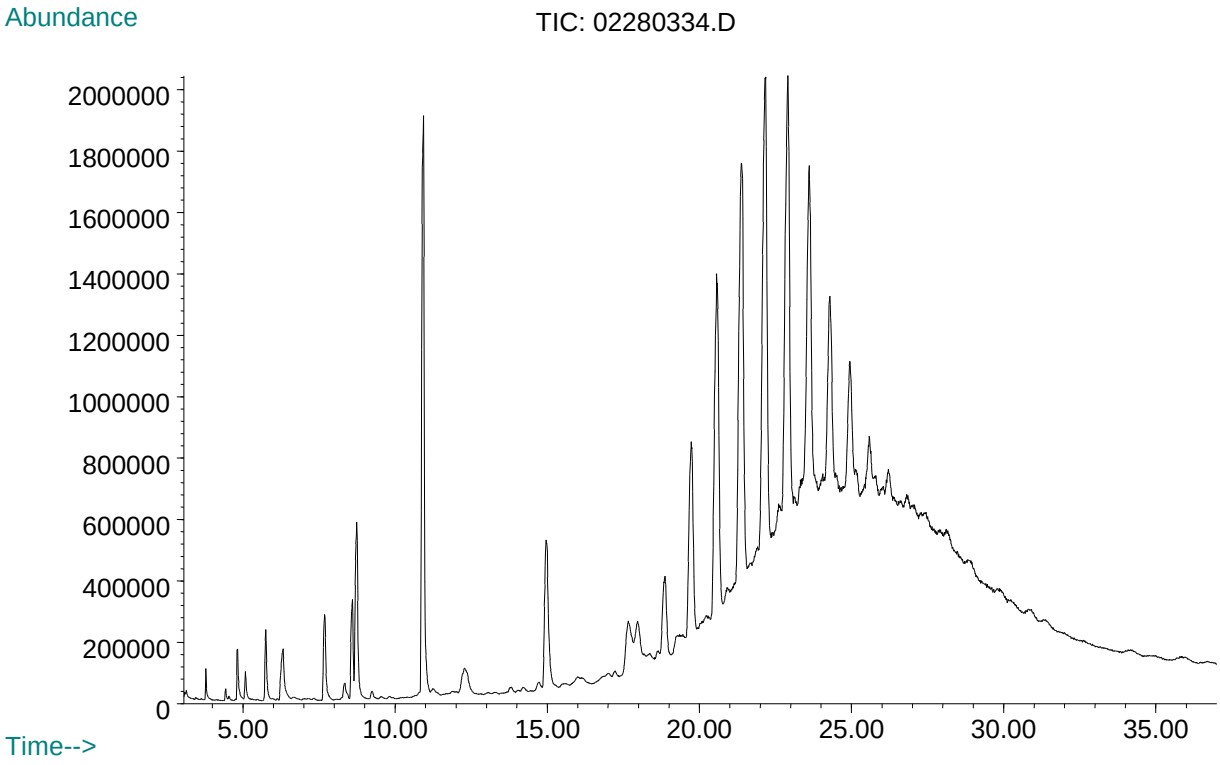


Figure 6. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the peroxide-cured elastomer test article, methylene chloride Soxhlet extract.

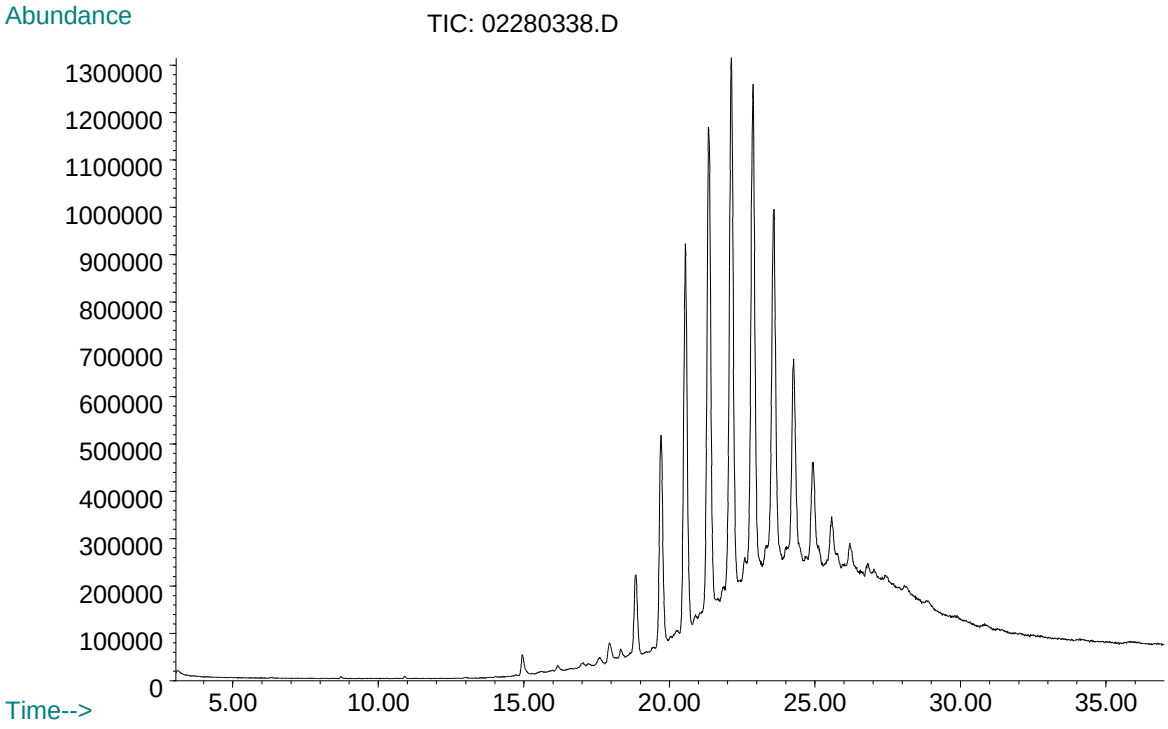


Figure 7. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the peroxide-cured elastomer test article, 2-propanol Soxhlet extract.

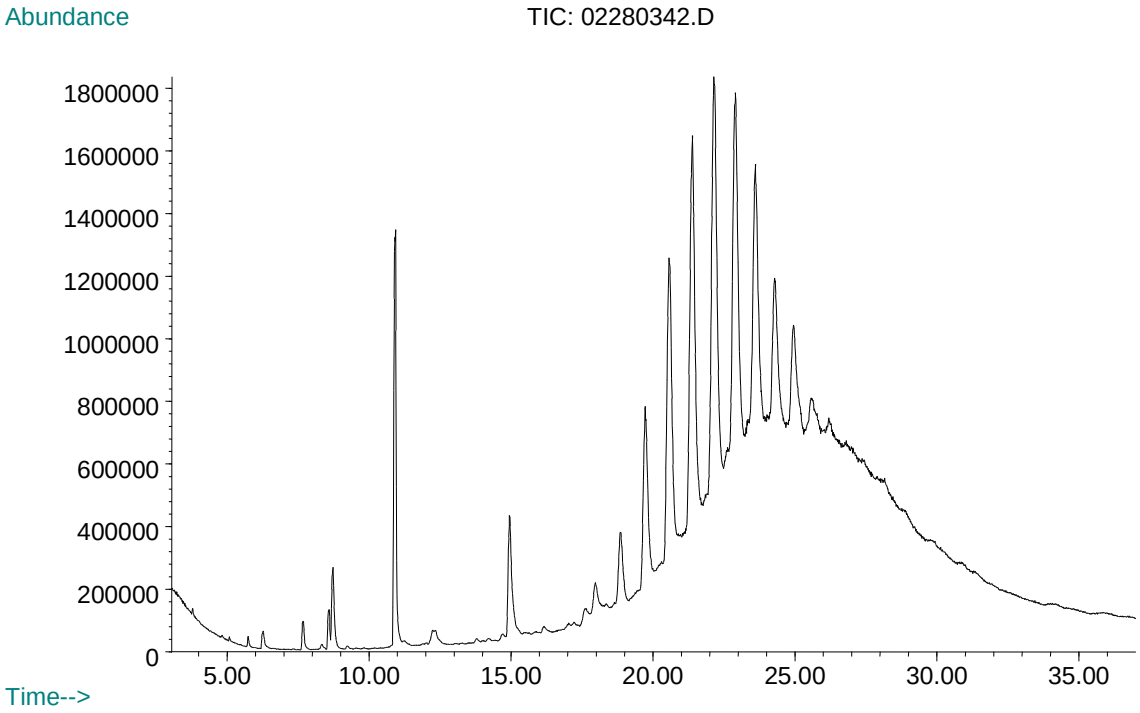


Figure 8. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the peroxide-cured elastomer test article, hexane Soxhlet extract.

The data provided in Figures 1-8 demonstrate that it is essential to conduct Controlled Extraction Studies using different solvents of varying polarity. In doing so, the pharmaceutical development team can determine an optimal solvent system that will produce a maximum number and concentration of extractables from a given test article, while complying with Jenke's directives. It is important to reiterate that the OINDP dosage form under development, the drug product formulation, and the type and composition of critical component materials should be taken into consideration when choosing solvents for Controlled Extraction Studies.

2. **Recommendation – Use of Multiple Extraction Techniques**

The Working Group chose to use Soxhlet extraction, sonication, and refluxing as extraction techniques in its laboratory Controlled Extraction Studies. These techniques were chosen because:

1. In the experience of Working Group members, these three techniques are, and have been, in common use in the industry for extractables studies and testing.

2. Each of these techniques has a long history of varied, safe and effective use in the scientific literature.

3. All three extraction techniques employ equipment which is routinely available in a typical analytical laboratory.

Experimental details for each extraction technique as applied to the different test articles are captured in the formal test protocols reproduced in Appendix 4. It is important to be aware that Controlled Extraction Studies for each of the test articles were accomplished in different volunteer laboratories. Although the use of a formal test protocol would serve to minimize interlaboratory variations in experimental procedures, such variations are inevitable in studies of this type and complexity. Recognizing this, the Working Group has drawn only the most general conclusions from the work, those least likely to be influenced by minor interlaboratory variability in experimental detail.

Figures 9, 10 and 11 show GC/MS extractables profiles (TICs) of extracts from sonication, Soxhlet and reflux of the sulfur-cured elastomer test article in 2-propanol. Note that on initial observation the number and intensity of peaks differ among extraction techniques, with Soxhlet and reflux appearing to be better than sonication. As noted previously, however, reflux in 2-propanol produced a potential artifact in the protic solvent mediated thermolysis of 2-mercaptobenzothiazole to benzothiazole. For the sulfur-cured elastomer and 2-propanol, Soxhlet would therefore appear to be the better choice of extraction technique.

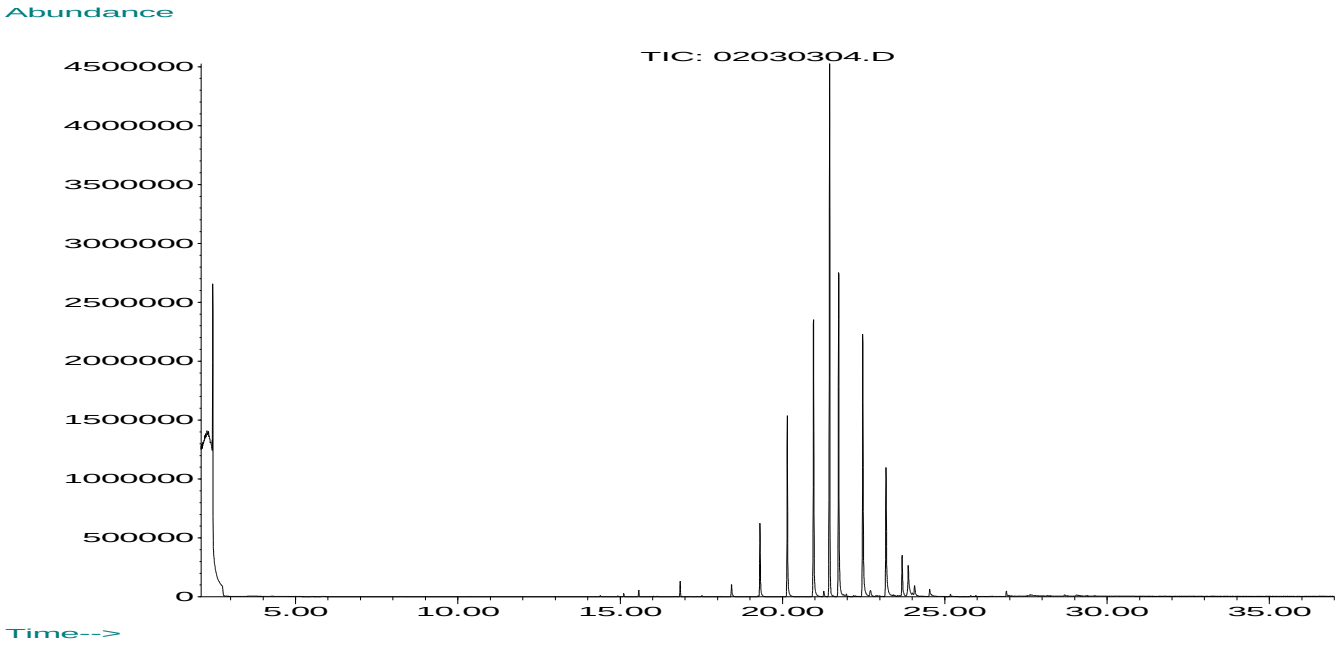


Figure 9. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the sulfur-cured elastomer test article, 2-propanol sonication extract. Sample reconstituted in methylene chloride prior to GC/MS analysis.

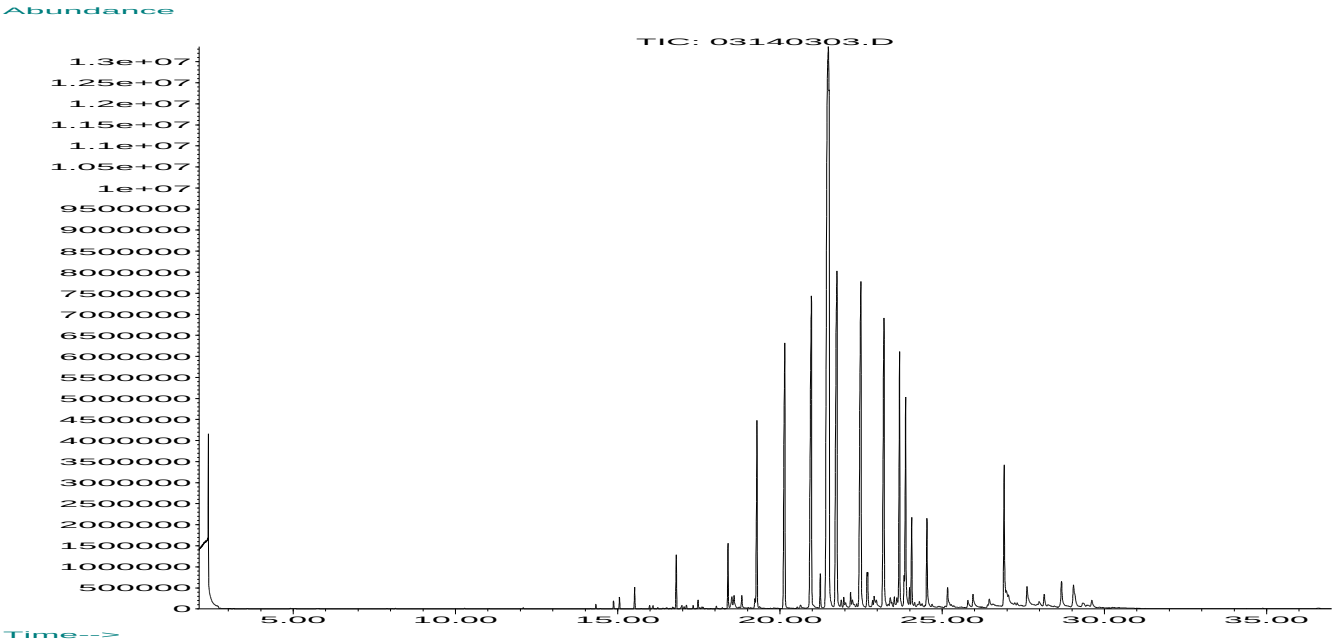


Figure 10. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the sulfur-cured elastomer test article, 2-propanol Soxhlet extract. Sample reconstituted in methylene chloride prior to GC/MS analysis.

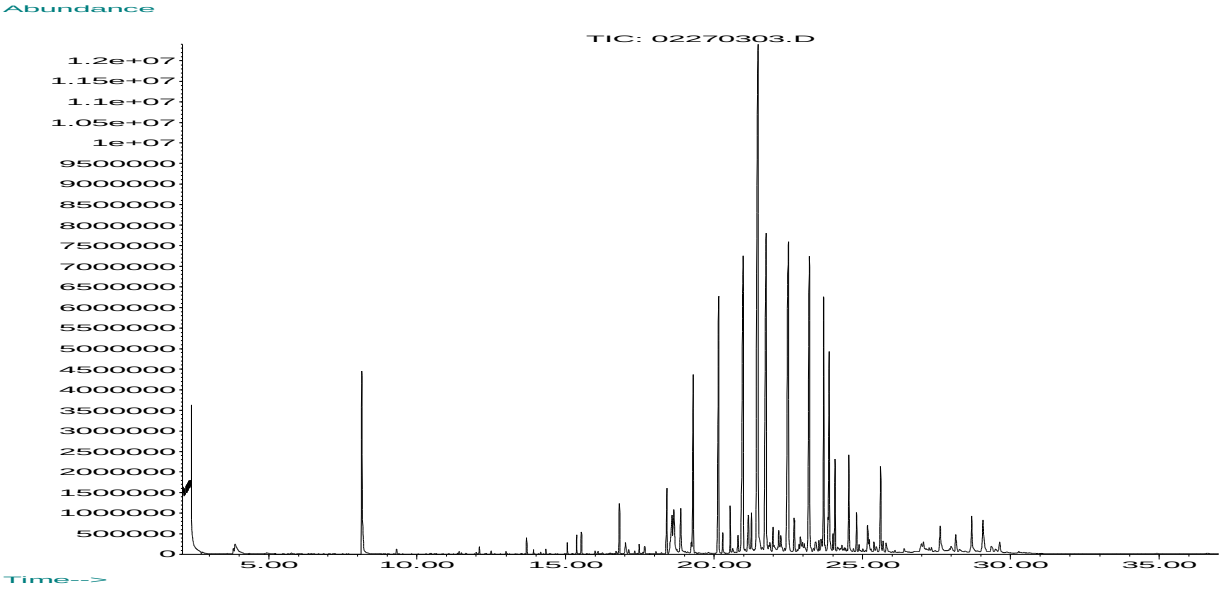


Figure 11. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the sulfur-cured elastomer test article, 2-propanol reflux extract. Sample reconstituted in methylene chloride prior to GC/MS analysis.

Figures 12 and 13 show GC/MS extractables profiles of the peroxide-cured elastomer test article using reflux and sonication with 2-propanol. Note the rather dramatic differences in the number and intensity of extractable peaks between the two extraction techniques.

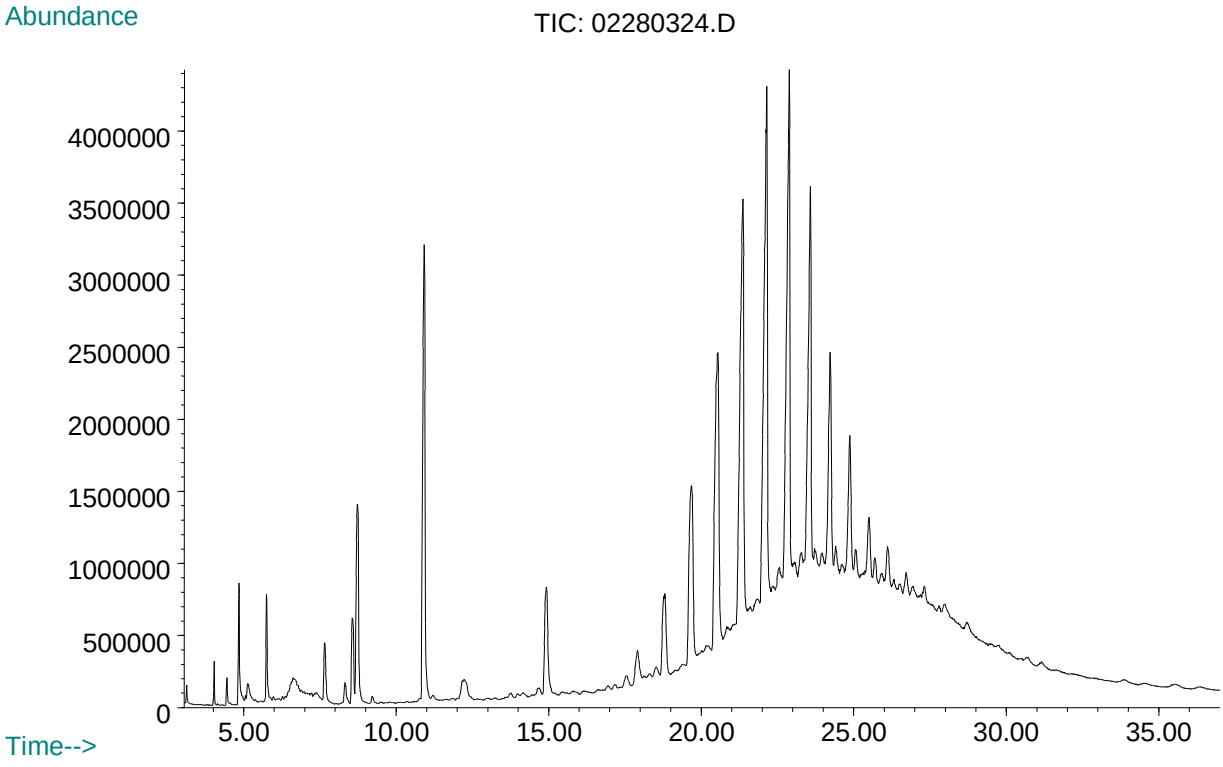


Figure 12. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the peroxide-cured elastomer test article, 2-propanol reflux extract.

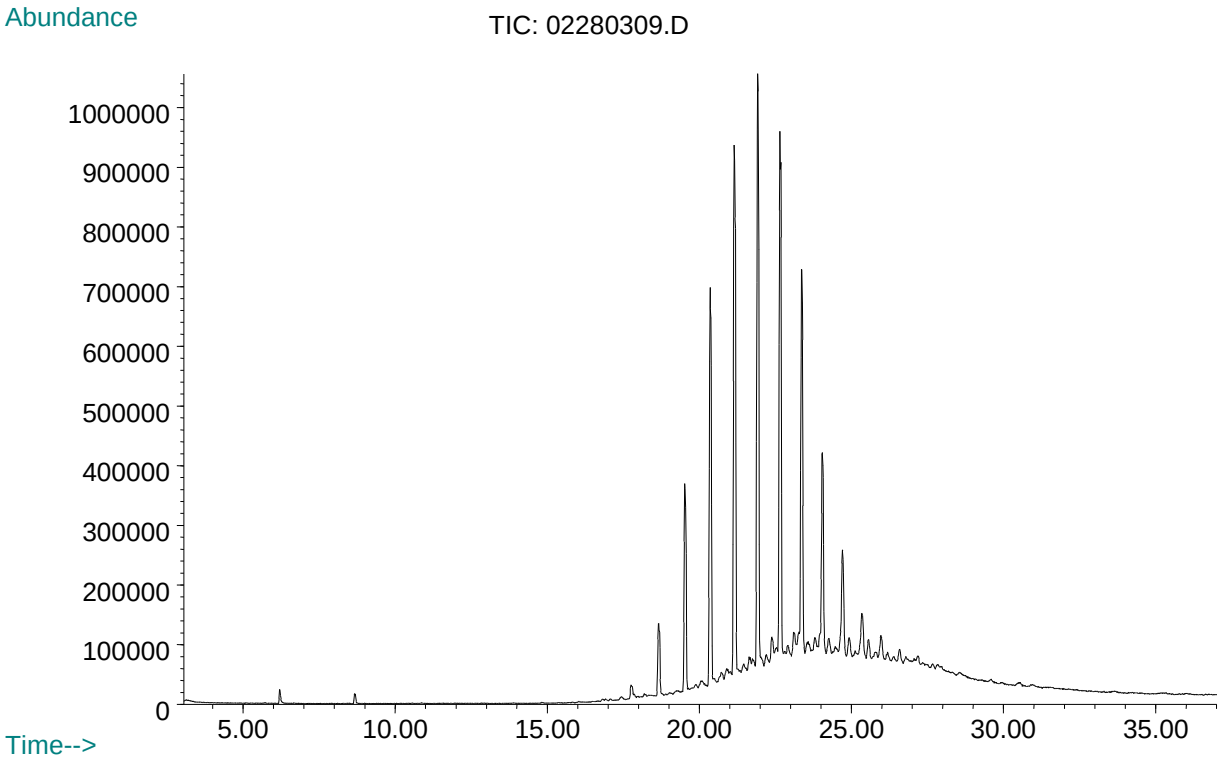


Figure 13. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the peroxide-cured elastomer test article, 2-propanol sonication extract.

Figures 14 and 15 show HPLC/DAD extractables profiles comparing reflux and sonication of the polypropylene test article with 2-propanol. Reflux yielded three significant extractable peaks representing the three known additives to the polypropylene formulation. Sonication yielded only one very small peak representing di-*tert*-butyl phenol derived from Ultranox 626. In addition to demonstrating the importance of assessing several different extraction techniques, these data show that for certain types of test article, certain extraction techniques are far more effective than others. In this case, it is clear that sonication was not useful in providing a comprehensive extraction of the polypropylene. The same conclusion might be drawn for the peroxide-cured elastomer.

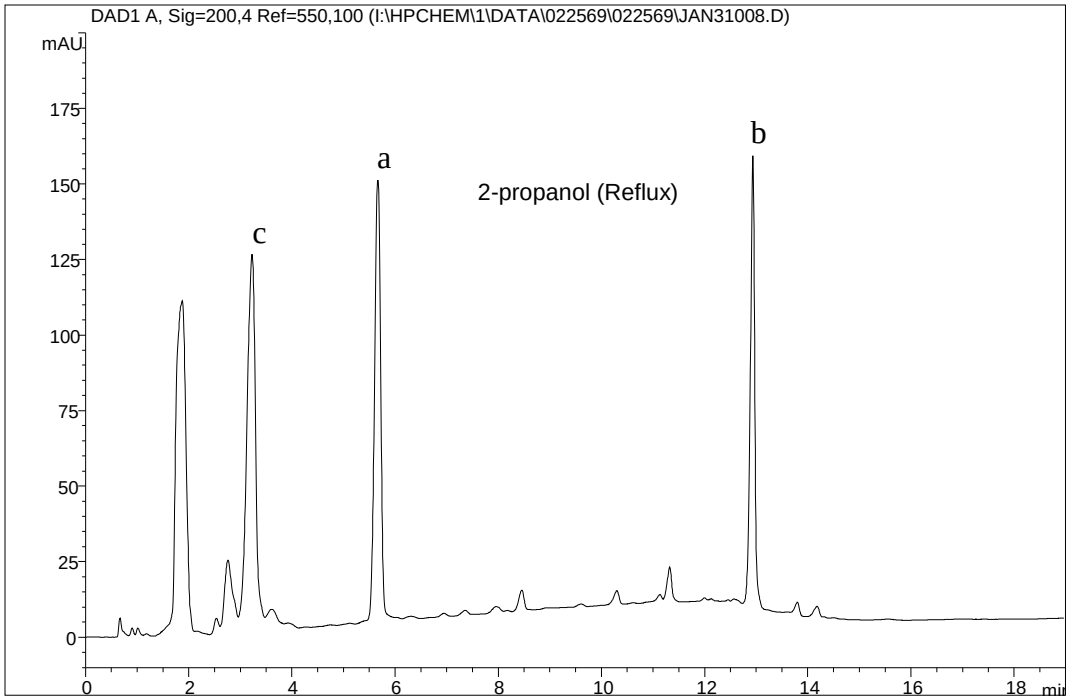


Figure 14. HPLC/DAD (High Performance Liquid Chromatography/Diode Array Detection) extractables profile (UV@200 nm) of a polypropylene test article 2-propanol reflux extract. **a** = di-*tert*-butylphenol from Ultranox 626; **b** = Tetrakis [methylene (3,5-di-*tert*-butyl-4-hydroxyhydrocinnamate)] methane; **c** = 3,4-dimethyl dibenzylidene sorbitol. The peak at approximately 2 minutes represents 2-propanol.

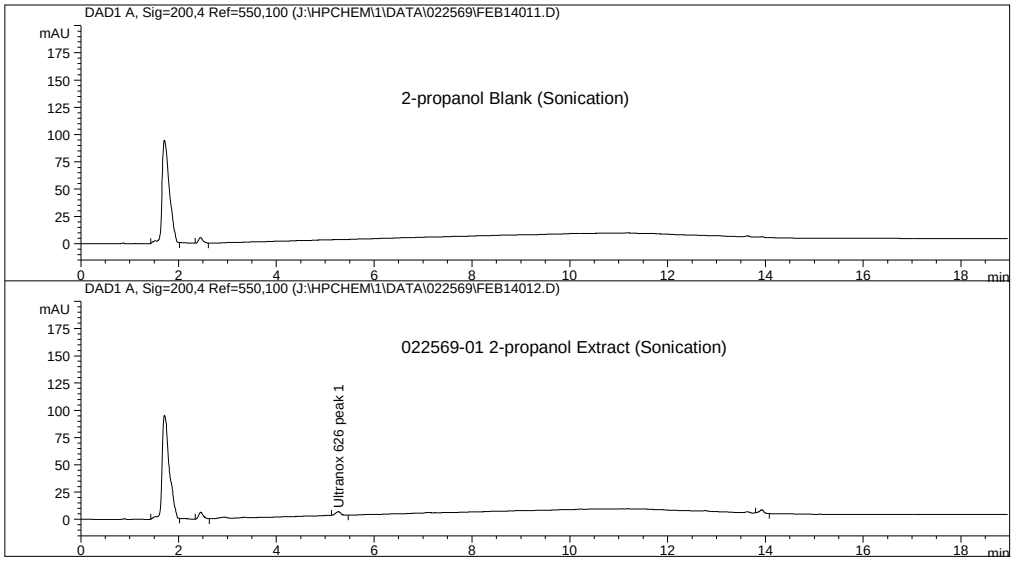


Figure 15. HPLC/DAD (High Performance Liquid Chromatography/Diode Array Detection) extractables profile (UV@200 nm) of a polypropylene test article 2-propanol sonication extract. The labeled peak at approximately 5.5 minutes is di-*tert*-butyl phenol from Ultranox 626.

At this point it is appropriate to briefly discuss the preparation of elastomer and plastic test articles for extraction in Controlled Extraction Studies. The Working Group believes that Controlled Extraction Studies are best accomplished on intact components. However, this does not preclude the use of additional sample preparation procedures (such as grinding or pressing in the case of plastic components), provided such procedures are justified and do not produce artifacts. For example, in some cases depending on the size and shape of the component, it may be more efficient to cut the sample into smaller, uniform pieces.

3. Recommendation - Effect of Sample Preparation

As stated previously, when using Gas Chromatography (GC) based analytical techniques, it is not always appropriate to inject high-boiling or reactive solvents, therefore it might be necessary to switch solvents prior to extractables profile analysis by either GC or GC/MS. Figures 16 and 17 show extractables profiles (TICs) of sulfur-cured elastomer extracts from reflux in 2-propanol. In Figure 16, 2-propanol was evaporated from the sample, and the sample was reconstituted in methylene chloride. Figure 17 shows results of neat, i.e., 2-propanol, sample injection. In this case, there appears to be no significant difference in results based on the sample preparation.

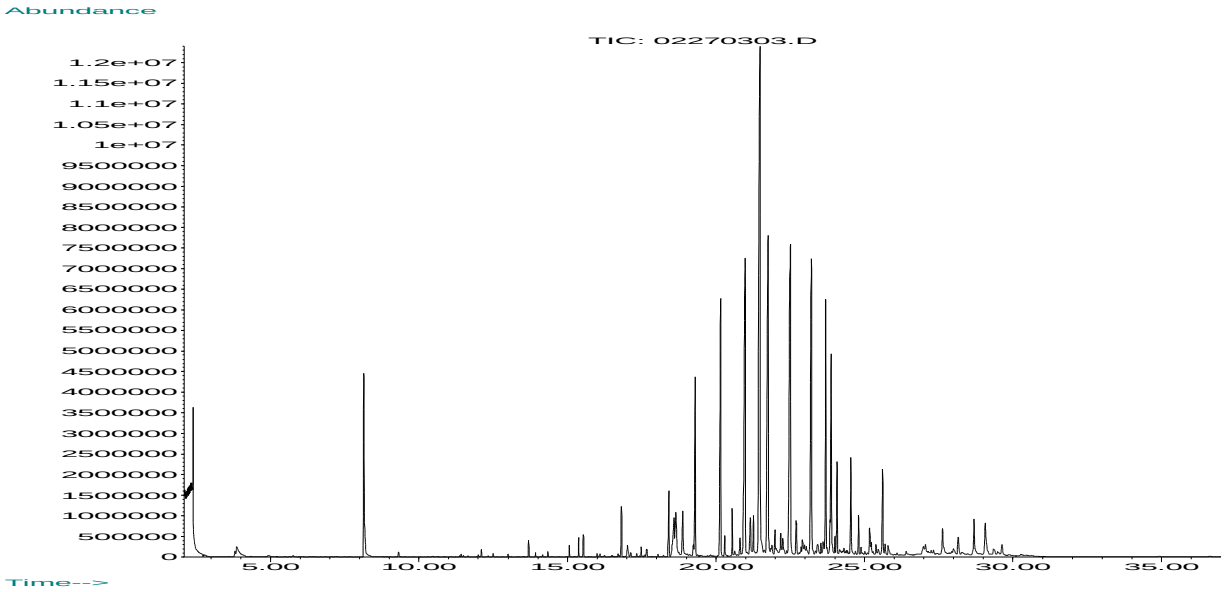


Figure 16. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the sulfur-cured elastomer test article, 2-propanol reflux extract. Sample reconstituted in methylene chloride prior to GC/MS analysis.

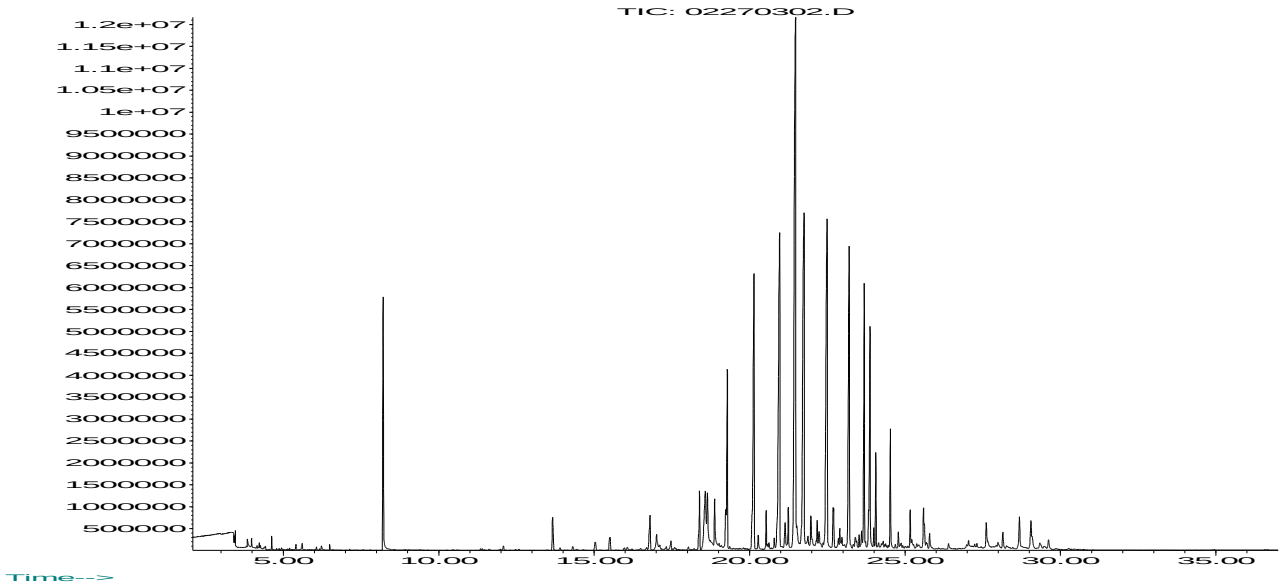
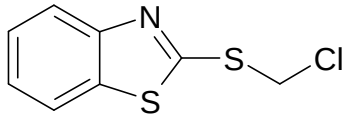


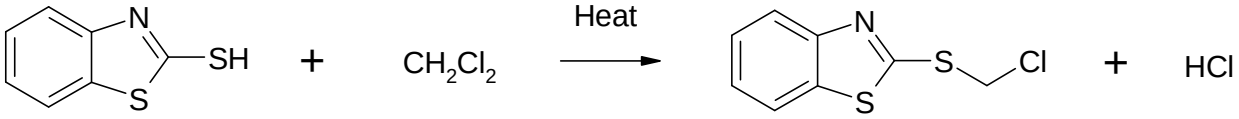
Figure 17. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the sulfur-cured elastomer test article, 2-propanol reflux extract. Sample injected neat, i.e., in 2-propanol.

However, an interesting "extractable" was observed in various methylene chloride extracts of the sulfur-cured elastomer:



2-(chloromethylthio)benzothiazole

This chemical entity was determined not to be an extractable from the sulfur-cured rubber, but is in fact a reaction product between methylene chloride and 2-mercaptobenzothiazole. This chemical reaction is likely promoted by the relatively high temperatures in the GC injector, and is clearly an artifact:



The analytical chemist should always be vigilant for extraction and analytical artifacts which could affect the interpretation of extractables profiles.

Also stated previously, when using Liquid Chromatography (LC) based analytical techniques it is usually inappropriate to inject samples contained in solvents which are not miscible in the liquid mobile phase. This is demonstrated by the HPLC/DAD extractables profiles in Figures 18 and 19. Figure 18 shows an extractables profile of polypropylene refluxed in methylene chloride and introduced neat to the HPLC system. Figure 19 shows an equivalent extractables profile of polypropylene refluxed in methylene chloride and then reconstituted in 1.0 mL of a 10:1 mixture of mobile phase A:B (where A = 75:25 acetonitrile/water, and B = 50:50 acetonitrile/THF) prior to HPLC sample introduction.

In Figure 18, the methylene chloride peak interferes significantly with the di-*tert*-butyl phenol peak, and completely obscures the 3,4-dimethyl dibenzylidene sorbitol (Millad 3988) peak. Peaks corresponding to these compounds are clearly visible in the chromatogram of Figure 19 (see Table 1 for complete peak identifications), where peak 2 corresponds to bis(dimethylbenzylidene) sorbitol isomer (from Millad 3988), peak 4 to di-*tert*-butylphenol (from Ultranox 626), and peak 12 to tetrakis [methylene (3,5-di-*tert*-butyl-4-hydroxyhydrocinnamate)] methane (Irganox 1010). Note also the relatively poor chromatographic performance apparent in Figure 18.

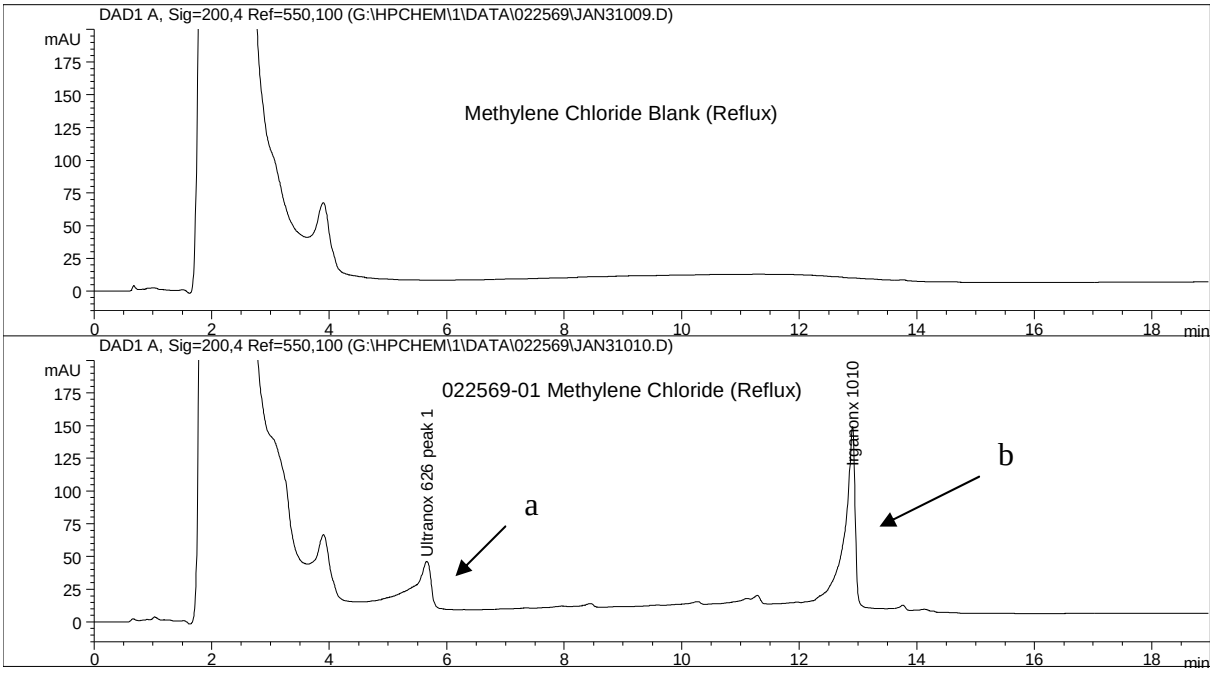


Figure 18. HPLC/DAD (High Performance Liquid Chromatography/Diode Array Detection) extractables profile (UV@200 nm) of a polypropylene test article methylene chloride reflux extract. Peak (**a**) = di-*tert*-butyl phenol from the Ultranox 626. Peak (**b**) = Irganox 1010.

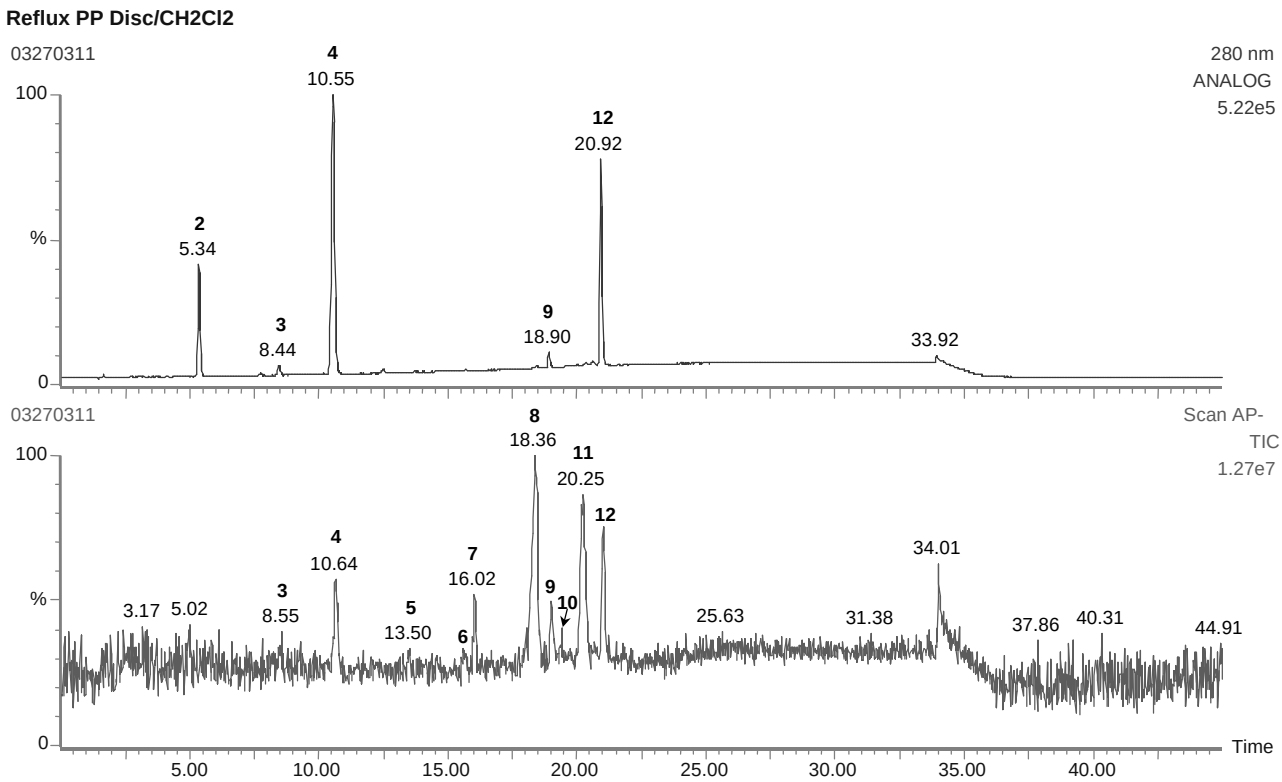


Figure 19. In-line HPLC/UV (@280 nm) and negative ion Total Ion Chromatogram (TIC) extractables profiles of a polypropylene test article methylene chloride reflux extract (see Table 1 for peak identifications). Note that the sample was injected onto the HPLC in a mobile phase mixture.

4. Recommendation – Use of Multiple Analytical Techniques

The Working Group used a variety of analytical techniques to detect, identify and quantify extractables in its Controlled Extraction Studies. These can be divided into broad classifications as shown below (see Appendix 4 for experimental and other details):

1. Techniques capable of detecting, identifying, and quantifying individual organic extractables:

    a. Gas Chromatography/Mass Spectrometry (GC/MS)

    b. Liquid Chromatography/Mass Spectrometry (LC/MS or HPLC/MS)

    c. Liquid Chromatography/Diode Array Detection (LC/DAD or HPLC/DAD)

2. Techniques capable of detecting and quantifying individual organic extractables:

    a. Gas Chromatography/Flame Ionization Detection (GC/FID)

b. Liquid Chromatography/Ultraviolet Detection (LC/UV or HPLC/UV)

3. Techniques capable of non-specific analysis of organic extract residues:

   a. Fourier Transform Infrared Spectroscopy (FTIR)

4. Techniques capable of detecting, identifying and quantifying inorganic extractables:

   a. Inductively Coupled Plasma/Mass Spectrometry (ICP/MS)

   b. Inductively Coupled Plasma/Optical Emission Spectroscopy (ICP/OES)

   c. Scanning Electron Microscopy/Energy Dispersive X-ray (SEM/EDX)

Results from the residue and inorganic extractables analytical work will not be discussed in this document. The recommendation detailed in the discussion below will focus on those analytical techniques most useful for detection, identification and quantification of individual organic extractables in Controlled Extraction Studies.

Any analytical technique useful for addressing Trace Organic Analysis problems must be capable of resolving complex mixtures of chemical entities, i.e., extractables and leachables, and detecting each entity individually[1,2]. The information obtained for each chemical entity must be directly related to, and interpretable based on, the molecular structure of the chemical entity. That is, the detection technique must be "compound specific". Further, in order to be quantitative, the response of the detector to any particular chemical entity must be directly proportional to either the absolute amount, i.e., mass or number of molecules, of the chemical entity or its concentration. The analytical techniques which best exhibit these attributes involve the combination of chromatography and mass spectrometry, GC/MS and LC/MS.

Extractables analyzed by GC/MS must be capable of entering the gas phase, i.e., volatilized, and pass through the separating GC column without chemical decomposition or irreversible adsorption. Further, each extractable must also be amenable to ionization by one of the ionization processes suitable for interface with GC/MS, the most commonly applied being electron ionization (EI) and chemical ionization (CI). The EI process involves the interaction of analyte molecules in the gas phase with an energetic beam of electrons, producing a radical cation (also termed the molecular ion or $M^{+\cdot}$). Excess internal energy in the molecular ion is distributed throughout its chemical bonds inducing fragmentation into smaller ions, each of which represents a portion of the original molecular structure of the extractable. Fragmentation processes can be interpreted from fundamental principles,[4,5] making it possible for an experienced analytical chemist to reassemble the original molecule from its fragment ions, i.e., interpret the mass spectrum. Since EI spectra are reproducible from instrument to instrument, it is also possible to search unknown EI spectra through databases (also called mass spectral libraries), providing a suitably informative EI spectrum can be obtained.

Chemical Ionization (CI)[6] involves the interaction of analyte molecules in the gas phase with an ionized gas, termed a "reagent or reactant gas". Ion-molecule collisions in the gas phase can result in proton transfer (or other) chemical reactions, producing so-called "protonated

molecular ions" ($[M+H]^+$) or other types of adduct ions, e.g., $[M+NH_4]^+$, when ammonia is used as a reagent gas. CI spectra are most useful for molecular weight confirmation, since CI is considered a "soft" ionization process resulting in little excess internal energy and fragmentation. EI and CI are therefore considered to be complementary. Note that although it is possible to acquire both positive and negative chemical ionization spectra (through proton abstraction or negative ion attachment processes), negative CI has very limited utility for extractables identification.

Extractables analyzed by LC/MS must be soluble in a liquid mobile phase and passed through the separating LC column without chemical decomposition or irreversible adsorption. Further, each extractable must also be amenable to ionization by one of the ionization processes suitable for interface with LC/MS, the most commonly applied being electrospray (ESI)[7] and atmospheric pressure chemical ionization (APCI).[8] In ESI, charged droplets of mobile phase containing analyte molecules and preformed ions, are evaporated in a strong electric field. The resulting highly charged droplets can desorb analyte protonated molecular ions and/or adduct ions (also deprotonated molecular ions and negative ion attachment ions) which can be collisionally stabilized in the gas phase. Extractables amenable to ESI are usually ionized in solution, and therefore ESI often reflects solution chemistry. APCI employs a corona discharge at atmospheric pressure to create an ionized reagent gas from mobile phase molecules. Ion-molecule reactions can then produce molecular ion species as with GC interfaced chemical ionization. Like CI, APCI is also a "soft" ionization process, with typically little fragmentation of molecular ion species. Because ESI and APCI (and also CI) involve collisions and ion-molecule reactions, these processes are said to be under thermodynamic control. Small variations in instrument parameters, such as reagent gas pressure and source temperature, can affect the appearance of these spectra making mass spectral libraries of limited value for unknown identification purposes. For extractables identification, positive and negative ESI and APCI spectra can be useful and complementary. It is also common practice to employ so-called "tandem mass spectrometry" or "MS/MS" techniques[9] to induce structurally useful fragmentation from molecular ions formed by "soft" ionization processes. Both GC/MS and LC/MS can also make use of accurate mass measurements, which enable elemental composition determinations and therefore reveal molecular formulas for unknowns. For more detailed discussion and review of GC/MS, LC/MS, and their application to the analysis of extractables and leachables, the reader is referred to Norwood, et al.[2]

The examples presented below give only a glimpse of the power of modern analytical chemistry. However, to quote Jenke,[3]

"The ability to compositionally characterize a delivery system by direct chemical/instrumental analysis remains a goal, rather than an accomplishment, of modern analytical chemistry."

In other words, there is no analytical technique or combination of techniques that can assure the absolute detection, identification and quantitation of all possible organic chemical entities that can appear as extractables in Controlled Extraction Studies. However, the concept of "due diligence" dictates that a pharmaceutical development team employ all appropriate and typically available analytical technologies to characterize OINDP component extractables profiles. Further, the instrumental parameters for both GC (GC/MS) and LC (LC/MS) employed for

extractables profiling in Controlled Extraction Studies should be as broad and general as possible. That is, the instrumental parameters, such as detection wavelength for LC/UV, GC temperature program parameters, and LC mobile phase elution power, should allow for the detection of a wide array of organic chemical compound classes and types.

(a) GC/MS and LC/DAD

The complementary nature of GC based and LC based analytical methods for use in Controlled Extraction Studies is appropriately illustrated with the polypropylene test article. The polypropylene was known to contain the antioxidant Irganox 1010, which a search of the available scientific literature revealed is most effectively analyzed by LC techniques. The structure of Irganox 1010 (chemical name: Tetrakis (methylene(3,5-di-*tert*-butyl-4-hydroxyhydrocinnamate)) methane) is shown below:

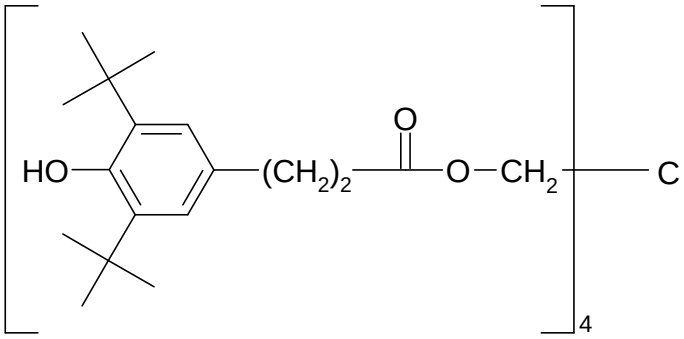


Figure 20 shows an expanded TIC from the GC/MS analysis of a 2-propanol reflux extract of the polypropylene test article. Note that due to its high molecular weight and lack of volatility, no intact Irganox 1010 was detected, and that there appears to be nothing in this extractables profile to suggest that Irganox 1010 is present in the extract.

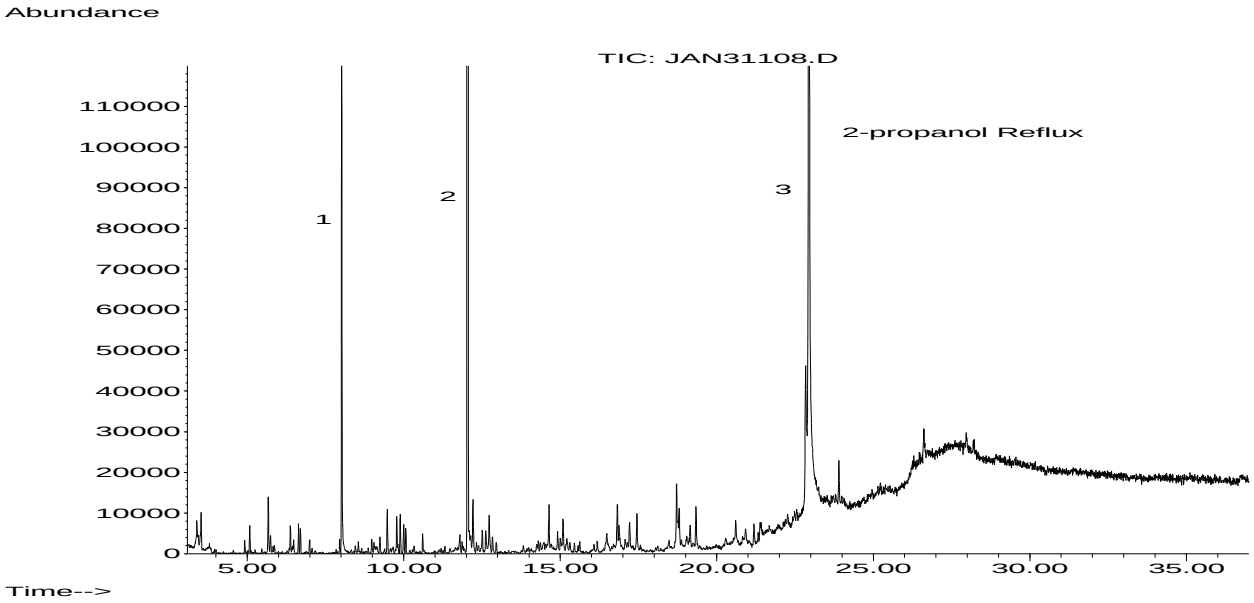


Figure 20. GC/MS analysis of extracts from reflux of polypropylene in 2-propanol. Peak 1 = 2,6-di-methyl benzaldehyde. Peak 2 = 2,4-di-*tert*-butylphenol. Peak 3 = glycerol monostearate.

Figure 2 shows an LC/DAD extractables profile from the same 2-propanol reflux extract which clearly shows the presence of Irganox 1010 as confirmed by UV spectrum and retention time match with an authentic standard. It is also important to note that LC/MS (ESI, APCI) in either positive or negative ion mode would have detected Irganox 1010 (see Figure 19). Most modern LCMS systems incorporate in-line DADs, so both UV and MS information are routinely available.

(b) **LC/UV and LC/MS**

Figure 21 shows the results of an LC/MS analysis (APCI in negative ion mode) of a methylene chloride reflux extract of the polypropylene test article. It is typical for LC/MS to have a UV or some other detector type in-line between the separating LC column and the mass spectrometer. The typical LC/MS analysis therefore, provides two chromatograms as shown in Figure 21, the top trace being a UV chromatogram at 280 nm and the bottom trace being an APCI negative ion Total Ion Chromatogram (TIC). It is readily apparent that several significant chromatographic peaks are present in the TIC which were not observed in the UV chromatogram, for example peaks 7, 8 and 11. The identities of these as well as other extractables detected in this extract, are given in Table 1. Note that peaks 7, 8, and 11 were identified respectively as:

Peak 7 Hexadecanoic acid (Palmitic acid)

Peak 8 Glycerol monopalmitate / Glycerol monostearate

Peak 11 Octadecanoic acid (Stearic acid)

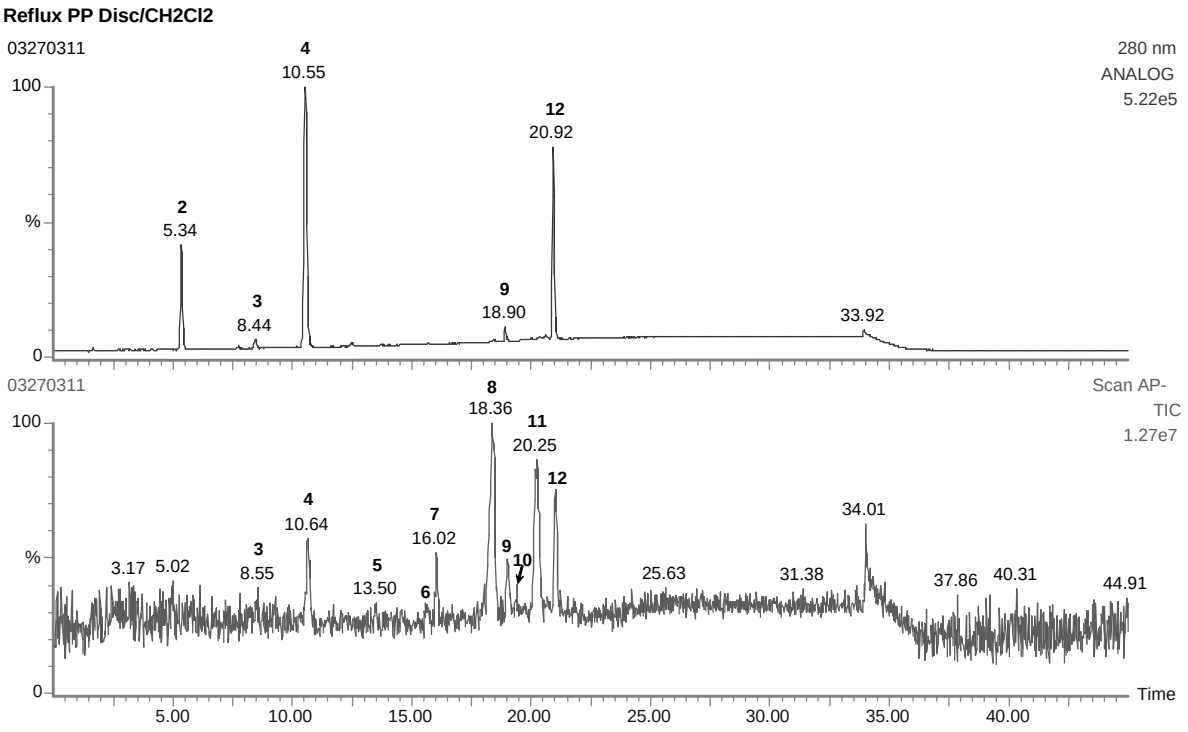


Figure 21. HPLC-UV chromatogram and negative ion Total Ion Chromatogram polypropylene disc, 4-hour methylene chloride reflux extract.

Table 1. Identifications of Extractables in a Methylene Chloride Reflux Extract of the Polypropylene Test Article from Analysis be Negative Ion APCI LC/MS with In-line UV Detection

Peak Number	Approximate Retention Time (min)	First Pass Identification
2	5.3	Bis(dimethylbenzylidene) sorbitol isomer
3	8.6	Unknown
4	10.6	Di-tert-butylphenol
6	15.6	Tetradecanoic acid
7	16.0	Hexadecanoic acid
8	18.4	Glycerol monopalmitate / Glycerol monostearate
9	19.0	Irganox 1010 fragment
10	19.4	Irganox 1010 related
11	20.3	Octadecanoic acid
12	21.0	Irganox 1010

Note that these particular extractables do not have chromophores in their molecular structures which would absorb at 280 nm. Therefore, in a first screening a wavelength range from, for example, 210 to 280 nm can be useful.

It is important to note that Total Ion Chromatograms produced from LC/MS analyses are typically dominated by mobile phase "cluster ions", which give the TIC an apparently poor signal-to-noise (note Figures 19 and 21). EI, on the other hand, has no such chemical background issues (see Figures 3 and 20, for example). It is common practice to use the in-line UV chromatogram (or chromatograms produced from other in-line detectors) along with so-called "mass chromatograms" or "extracted ion current profiles" to locate peaks in a total ion chromatogram (note Figures 22-23 below). Figure 22 shows the in-line UV chromatogram (top trace) and an extracted ion chromatogram for m/z 1175 which is the $[M-H]^-$ for Irganox 1010 (see Figure 23).

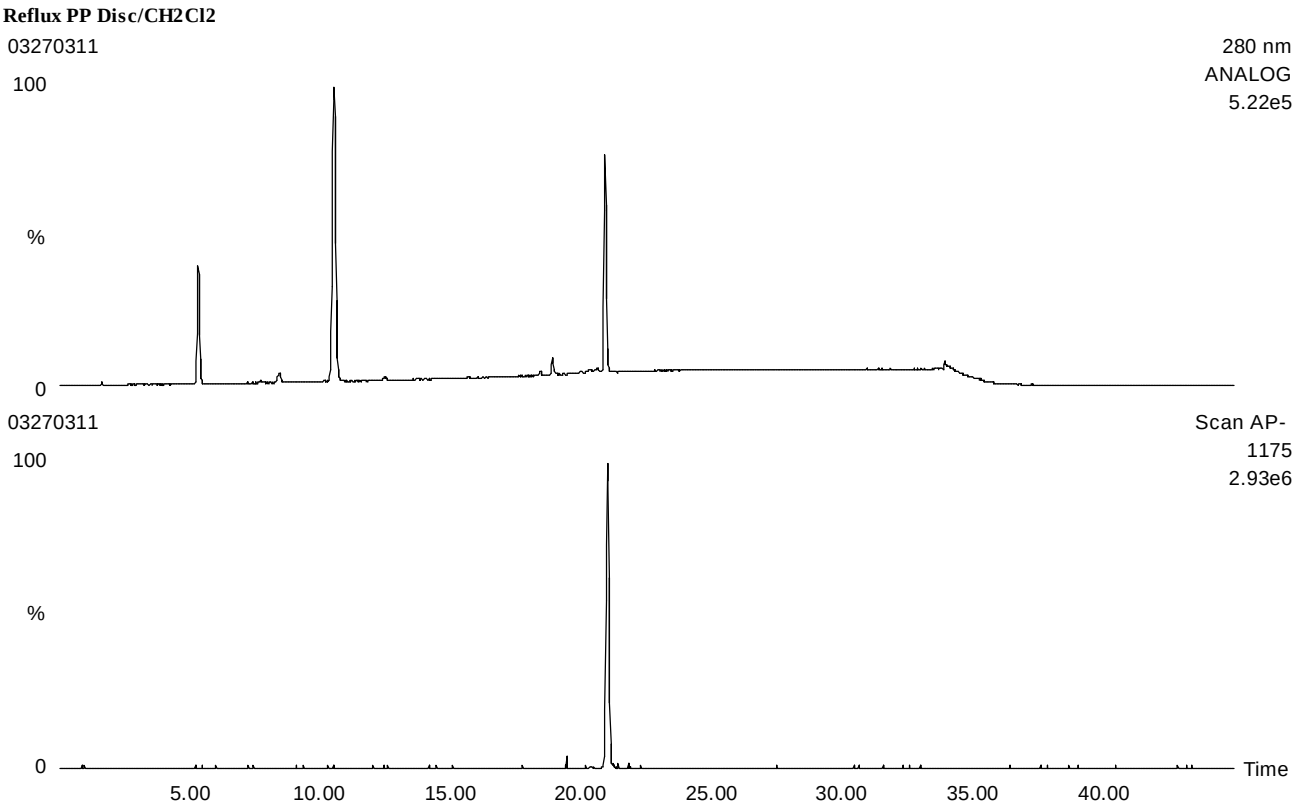


Figure 22. HPLC-UV chromatogram and m/z 1175 extracted ion current profile polypropylene disc, 4-hour methylene chloride reflux extract.

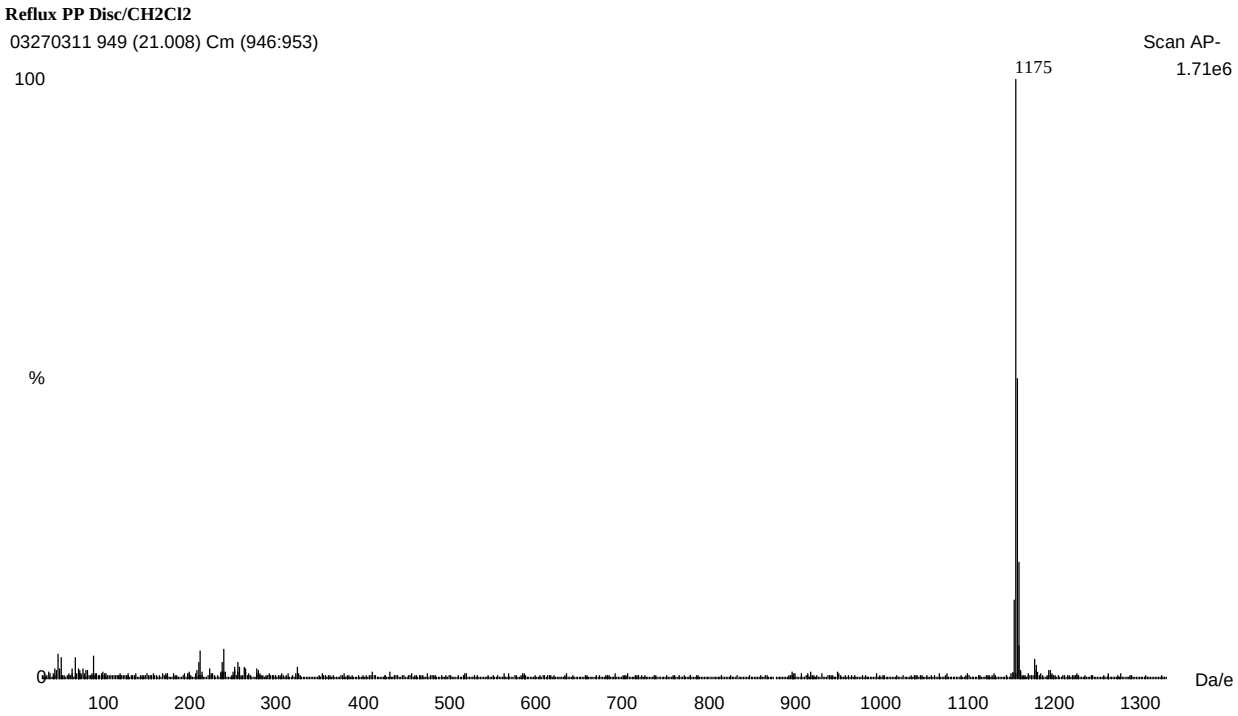
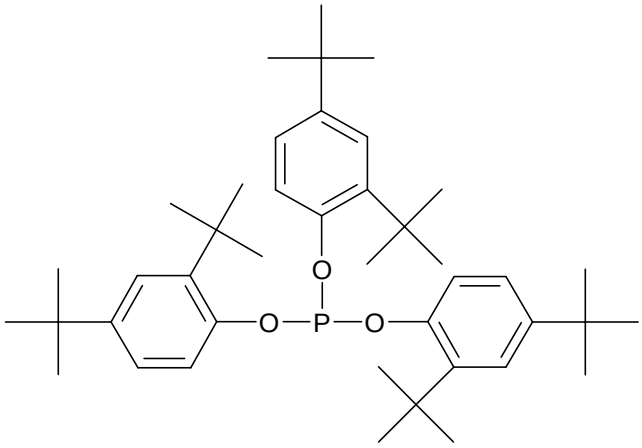


Figure 23. Negative ion APCI mass spectrum of Irganox 1010. Note the $[M-H]^-$ at m/z 1175.

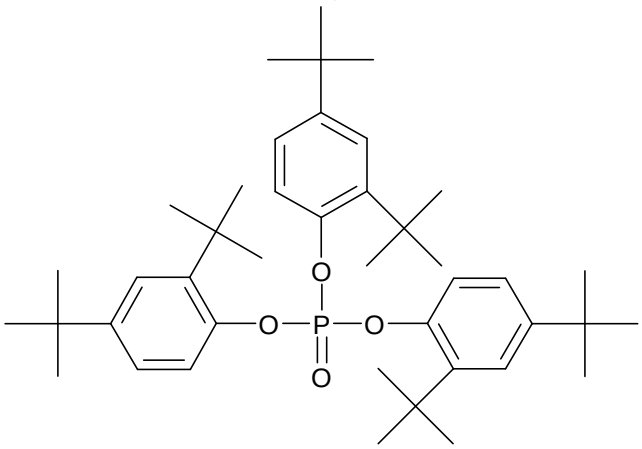
LC/MS analysis in positive ion mode can be equally complementary as shown in Figure 24. Note that in the TIC from the positive ion APCI LC/MS analysis (bottom trace in Figure 24), several extractables are apparent which were not detected in either the corresponding UV chromatogram or in the negative ion APCI LC/MS analysis. For example, peak 14 was identified as Tris(2,4-di-*tert*-butylphenyl) phosphate (**II**) which is likely related to the trivalent phosphorus antioxidant Irgafos 168 (**I**) by the following oxidation reaction:

(I) Irgafos 168



I

(oxidizing agent)



II

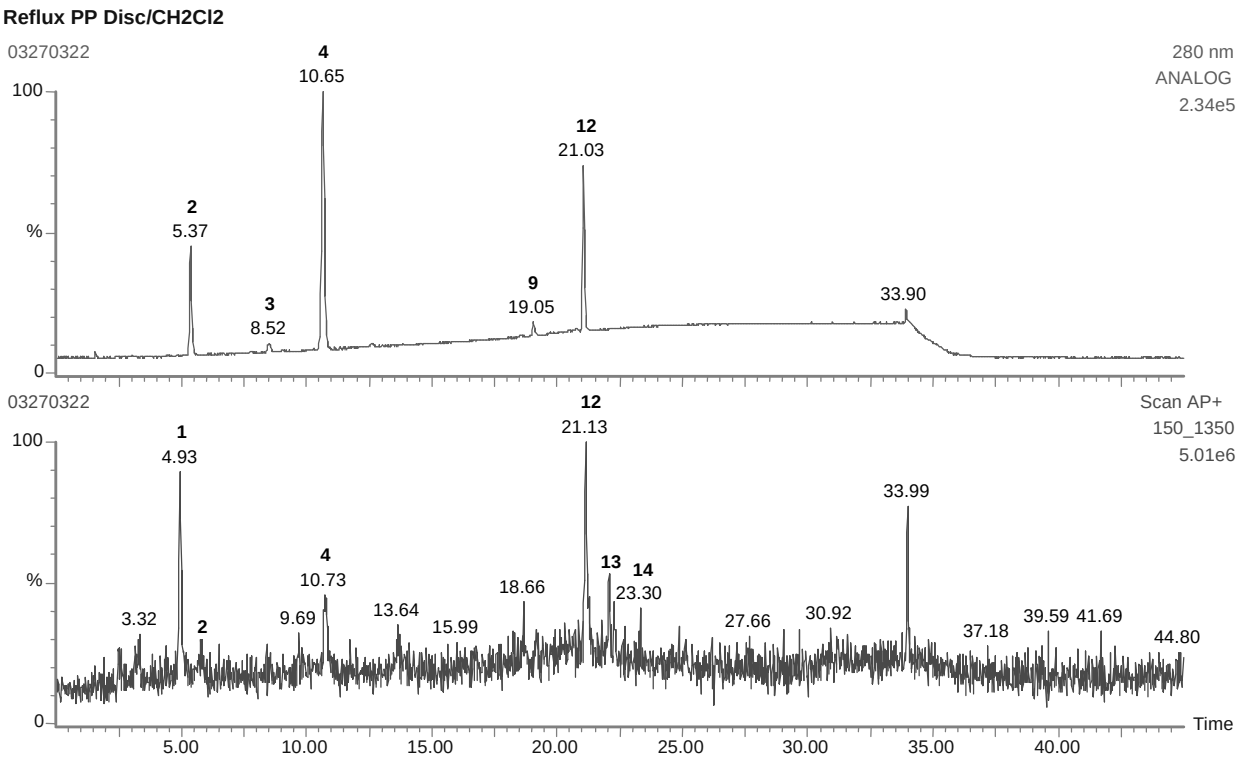


Figure 24. HPLC-UV chromatogram and positive ion Total Ion Chromatogram polypropylene disc, 4-hour methylene chloride reflux extract.

These are but a few of many examples of complementary analytical techniques that the Working Group discovered during its model Controlled Extraction Studies. It is clearly the case that in order to ensure complete characterization of OINDP component extracts during Controlled Extraction Studies, the use of multiple analytical techniques is required. Analytical technique selection should be guided by what is known about the composition of a particular component and sound scientific practice. For additional information, discussion, and review the reader is referred to other works of Jenke.[10,11]

5. Recommendation – Comprehensive/Systematic Identification of Extractables

As demonstrated by the representative data depicted and discussed thus far in this chapter, extractables profiles acquired during Controlled Extraction Studies can be highly complex. For example, a comprehensive evaluation of GC/MS extractables profiles from the sulfur-cured elastomer test article (Figure 25, for example) determined that 66 individual chemical entities were detected as extractables. Many of these extractables were related to the Coumarone-indene resin natural product material used in the elastomer recipe. Given the number and chemical nature of extractables from this material, it is not reasonable to expect that authentic reference compounds will be available (or can be made available) to confirm every identification. It is therefore both reasonable and necessary that additional levels of identification confidence by established and appropriately utilized.

Any successful process used for identification, i.e., elucidation of molecular structure, of individual extractables (and leachables) must be comprehensive and systematic. The data and

interpretation processes used for each identification must be clearly defined and understood. An example of such a systematic process for GC/MS and LC/MS extractables profile evaluation, based on a similar proposal for identification of trace level organic compounds in environmental samples,[12] is presented in Table 2 and discussed below. In Table 2, data typically available from GC/MS and LC/MS analyses are assigned "Identification Categories," which are used to designate individual extractables identifications as *Confirmed*, *Confident*, or *Tentative*. An application of this process to a GC/MS extractables profile from the sulfur-cured elastomer test article is shown in Table 3.

Table 2. Identification Categories for Structure Elucidation of Extractables and Leachables by GC/MS and LC/MS

Category	Supporting Identification Data
A	Mass spectrometric fragmentation behavior
B	Confirmation of molecular weight
C	Confirmation of elemental composition
D	Mass spectrum matches automated library or literature spectrum
E	Mass spectrum and chromatographic retention index match authentic specimen

- A *Confirmed* identification means that identification categories A, B (or C), and D (or E) have been fulfilled.
- A *Confident* identification means that sufficient data to preclude all but the most closely related structures have been obtained.
- A *Tentative* identification means that data have been obtained that are consistent with a class of molecule only.

Table 3. Extractables Identified from the GC/MS Analysis of a Methylene Chloride Soxhlet Extract of the Sulfur-Cured Elastomer Test Article (see Figure 25)

Peak #	Identification	Retention Time (min)	Identification Categories	Identification Level
1	α-Methylstyrene	4.90	A, C, D, E	Confirmed
2	Indene	5.70	A, C, D, E	Confirmed

3	Naphthalene	7.65	A, B, D	Confirmed
4	Tetramethylthiourea	8.05	A, C, D, E	Confirmed
5	Benzothiazole	8.15	A, C, D, E	Confirmed
6	Ethyl-4-tert-butyl phenyl ether	11.05	A, D	Confident
7	2,5-di-tert-butylphenol	12.10	A, D	Confident
8	2-(methylthio) benzothiazole	12.86	A, D	Confident
9	Coumarone-indene resin related	14.35	A, C, D	Confirmed
10	2-(chloromethylthio) benzothiazole	14.86	A, circumstantial	Confident
11	Coumarone-indene resin related	15.05	A, C, D, E	Confirmed
12	Coumarone-indene resin related	15.52	A, C, D	Confirmed
13	Coumarone-indene resin related	15.97	A, C	Tentative
14	Coumarone-indene resin related	16.07	A, C	Tentative
15	Coumarone-indene resin related	16.24	A, C	Tentative
16	2-mercaptobenzothiazole	16.40	A, C, D	Confirmed
17	Coumarone-indene resin related	16.80	A, C	Tentative
18	Hexadecanoic acid	16.98	A, C, D, E	Confirmed
19	3,5-bis-(1,1-dimethylethyl-4-hydroxy) benzoic acid	17.04	A, D	Confident
20	Isomer of peak 19	17.11	A, D	Confident
21	Coumarone-indene resin related	17.31	A	Tentative
22	n-Eicosane	17.47	A, D	Confident
23	bis-(4-methylphenyl) disulfide	17.53	A, D	Confident
24	Unknown (possible coumarone-indene resin related)	18.03	A	Tentative
25	Heneicosane	18.39	A, B, D, E	Confirmed
26	Linoleic acid	18.52	A, D	Confident
27	(E)-octadecenoic acid	18.60	A, D	Confident
28	Stearic acid	18.84	A, C, D, E	Confirmed
29	1-octadecene	19.22	A, D	Confident
30	n-Docosane	19.28	A, B, D, E	Confirmed
31	Tricosane	20.12	A, B, D, E	Confirmed
32	Unknown (MW 366)	20.53	-	-
33	Tetracosane	20.94	A, B, D, E	Confirmed
34	Coumarone-indene resin related	21.24	A, C	Tentative
35	2, 2'-methylene-bis-(-6-tert-butyl)-4-ethylphenol	21.47	A, B, D, E	Confirmed
36	Pentacosane	21.73	A, B, D, E	Confirmed
37	Coumarone-indene resin related	21.88	A, C	Tentative
38	Unknown (possible coumarone-indene resin related)	21.96	A	Tentative
39	n-alkane	22.17	A	Tentative
40	unknown	22.24	-	-
41	Hexacosane	22.48	A, B, D, E	Confirmed
42	Coumarone-indene resin related	22.68	A, C	Tentative

43	Coumarone-indene resin related	22.71	A, C	Tentative
44	Coumarone-indene resin related	22.86	A, C	Tentative
45	Heptacosane	23.20	A, B, D	Confirmed
46	Coumarone-indene resin related	23.40	A, C	Tentative
47	Coumarone-indene resin related	23.45	A, C	Tentative
48	Coumarone-indene resin related	23.53	A, C	Tentative
49	Coumarone-indene resin related	23.68	A, C	Tentative
50	Coumarone-indene resin related	23.80	A, C	Tentative
51	Octacosane	23.88	A, B, D, E	Confirmed
52	Coumarone-indene resin related	23.99	A, C	Tentative
53	Coumarone-indene resin related	24.06	A, C	Tentative
54	Coumarone-indene resin related	24.15	A, C	Tentative
55	Nonacosane	24.54	A, B, D, E	Confirmed
56	Triacontane	25.17	A, D, E	Confident
57	n-alkane	25.80	A	Tentative
58	β-Sitosterol	26.93	A, D	Tentative
59	Coumarone-indene resin related	27.05	A	Tentative
60	Coumarone-indene resin related	27.63	A, C	Tentative
61	Coumarone-indene resin related	28.01	A	Tentative
62	Coumarone-indene resin related	28.16	A	Tentative
63	Coumarone-indene resin related	28.69	A, C	Tentative
64	Coumarone-indene resin related	29.07	A, C	Tentative
65	Coumarone-indene resin related	29.35	A, C	Tentative
66	Coumarone-indene resin related	29.63	A, C	Tentative

3099

3100

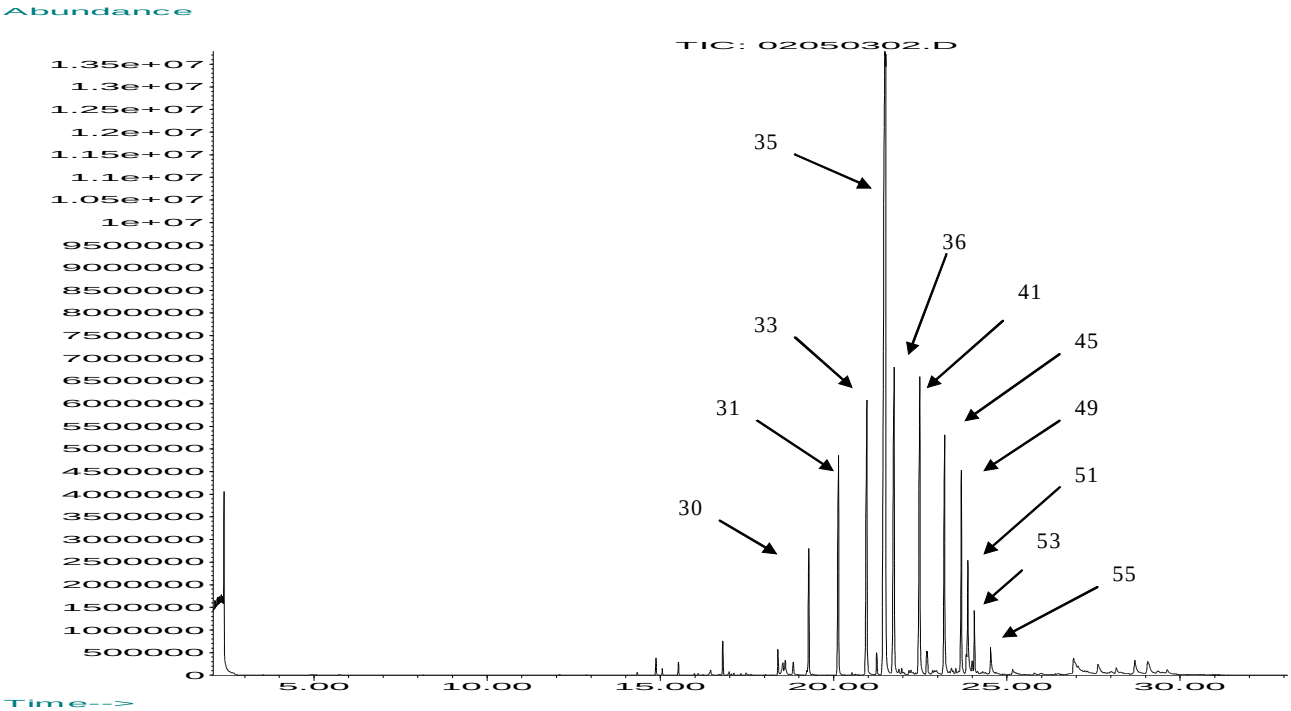


Figure 25. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the sulfur cured elastomer test article, methylene chloride Soxhlet extract.

Figures 26-29 provide examples of data from the *Confirmed*, *Confident*, and *Tentative* identifications in Table 3. Figure 26 provides an example of confirmation of molecular weight of a compound (Category B in Table 2). The figure shows chemical ionization (CI; ammonia reagent gas) and electron ionization (EI) mass spectra of peak #35. Note that the $[M+NH_4]^+$ at m/z 386 in the CI mass spectrum confirms the likely molecular ion in the EI mass spectrum at m/z 368. The monoisotopic molecular weight of this extractables is, therefore, 368 amu. In addition to this information, fragmentation behavior, mass spectral library match, and retention time match with an authentic standard confirmed this compound as 2, 2'-methylene-bis-(-6-*tert*-butyl)-4-ethylphenol.

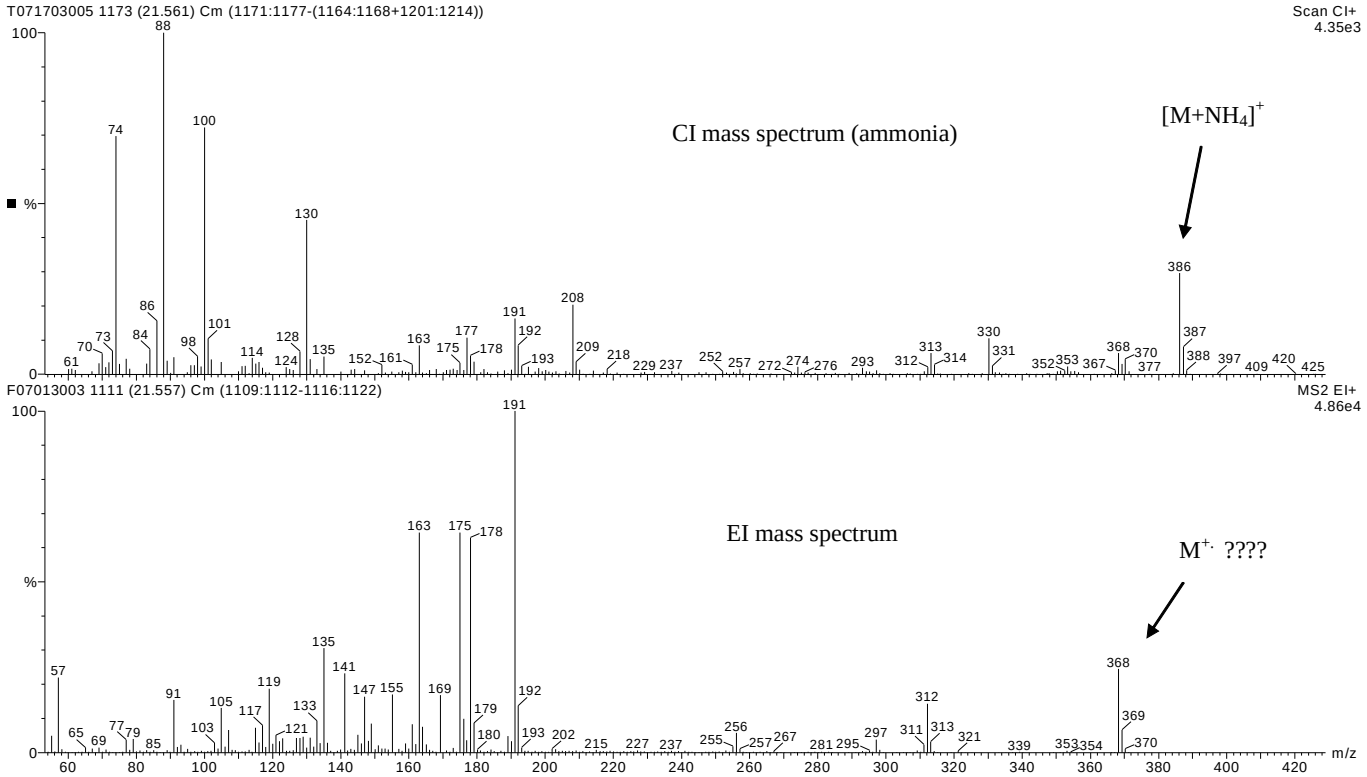


Figure 26. Ammonia chemical ionization (CI) mass spectrum (top) and electron ionization (EI) mass spectrum (bottom) of 2,2'-methylene-bis-(-6-*tert*-butyl)-4-ethylphenol (peak #35 in Table 3). Note that the $[M+NH_4]^+$ at m/z 386 in the CI spectrum confirms m/z 368 in the EI spectrum as the molecular ion ($M^{+\cdot}$), and therefore represents the molecular weight of the extractable.

Figure 27 shows an example of a positive mass spectral library match (Category D in Table 2) between EI spectra from peak #5 and benzothiozole. Other information such as fragmentation behavior, retention time match with authentic standard and elemental composition, confirmed this compound as benzothiozole.

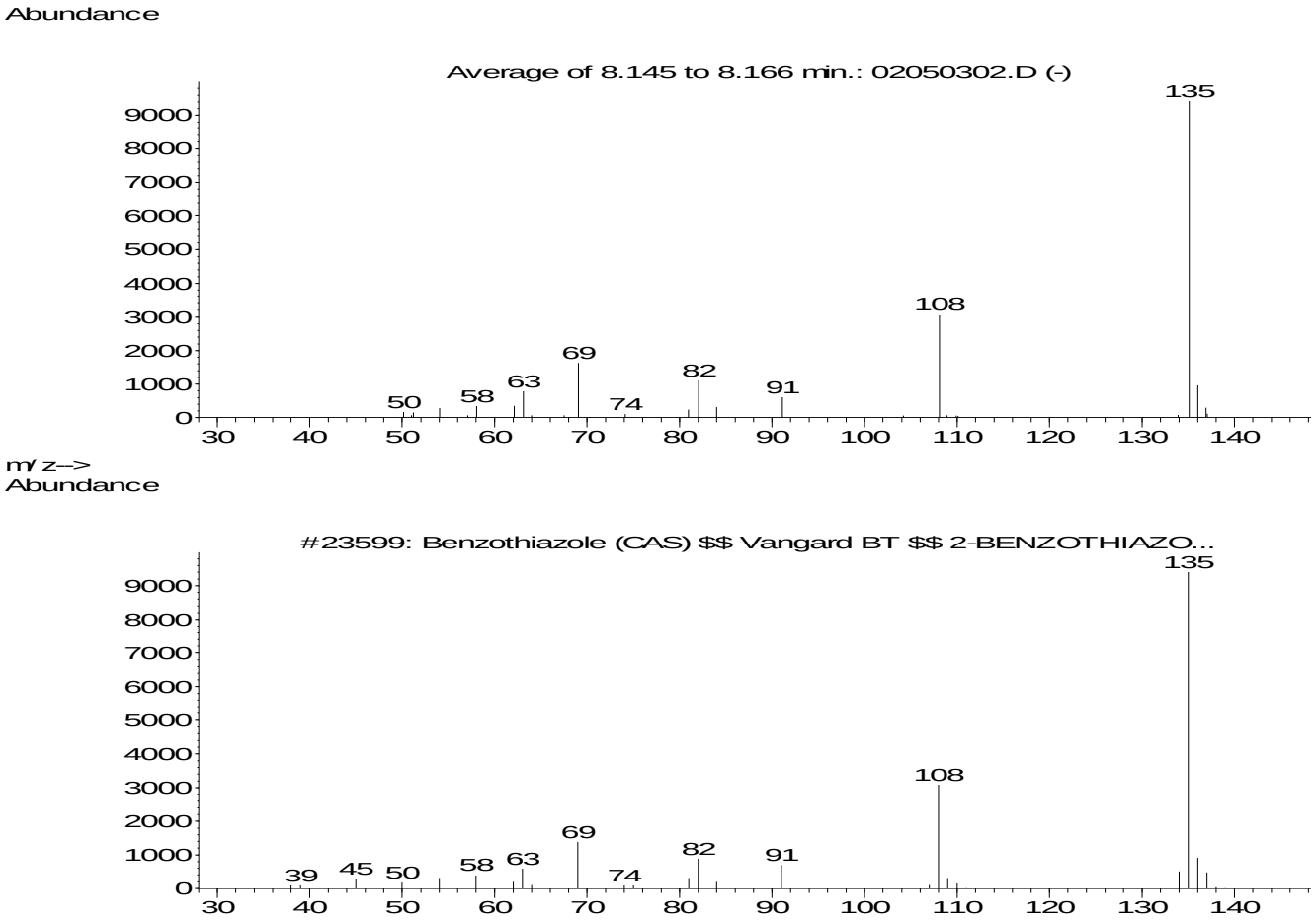


Figure 27. Electron ionization (EI) mass spectrum of extractable peak #5 from Table 3 (top) with best fit mass spectral library match (bottom) or benzothiazole.

Figures 28 and 29 provide an example of evaluation of the fragmentation behavior and confirmation of elemental composition of compound #11 from Table 3 (Categories A and C from Table 2). Figure 28 shows the EI mass spectrum of peak #11. The measured accurate mass of the molecular ion (m/z 236) suggested a likely molecular formula of m/z 236 $C_{18}H_{20}$ (2.7 ppm accurate mass measurement). Plausible structures were proposed for the major fragment ions in the mass spectrum as shown in Figure 29. Additional information allowed confirmation of this compound as a derivative of the coumarone-indene resin.

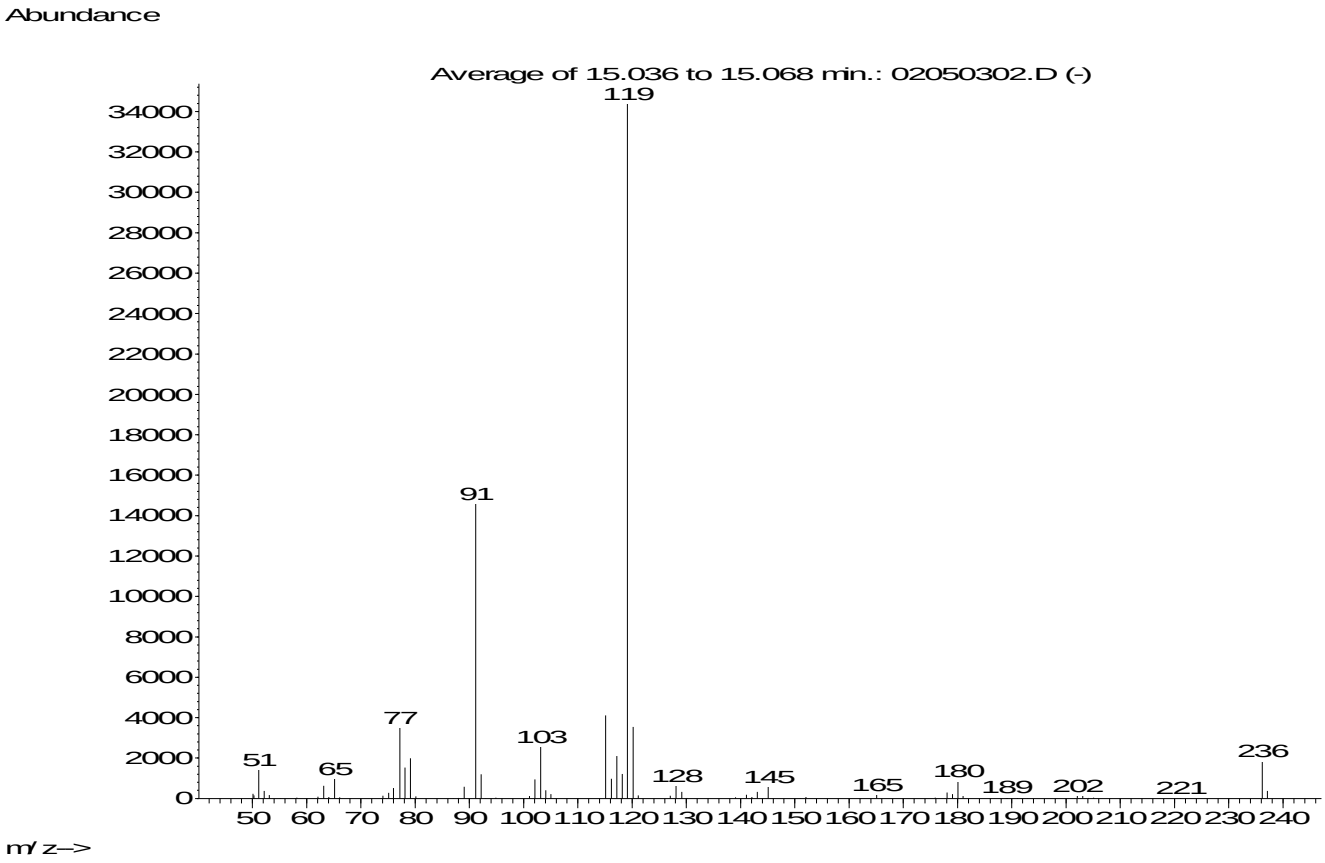
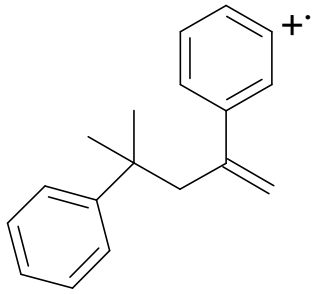
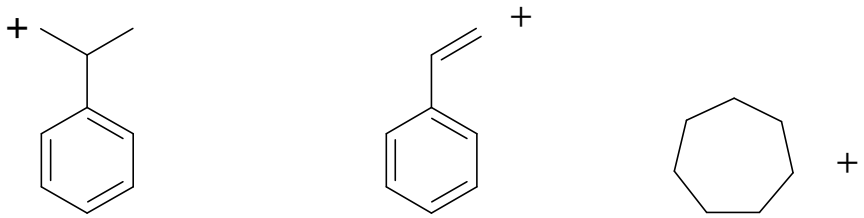


Figure 28. Electron ionization mass spectrum of peak #11 in Table 3.

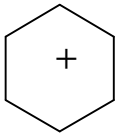


m/z 236

$C_{18}H_{20}$ (2.7 ppm accurate mass measurement)



m/z 119 m/z 103 m/z 91 (tropylium ion)



m/z 77 (benzylium ion)

Figure 29. Structures for major fragment ions in the EI mass spectrum for peak #11.

6. Recommendation - Optimization and Quantification

As stated above, after evaluating extractables profiles from various extraction techniques/methods and solvents, a pharmaceutical development team should choose a "definitive" extraction technique(s)/method(s) to optimize. An optimized extraction method is defined as one that yields a high number and concentration of extractables, e.g., steady-state or "asymptotic levels," without violating Jenke's directives. This is not meant to imply that 100% of all known additives must be recovered. Optimization of the extraction technique(s)/method(s) prior to conducting quantitative Controlled Extraction Studies ensures that the extractables profile(s) represents at least a "worst-case" scenario of potential leachables and their levels. Extractables profiles produced from such optimized technique(s)/method(s) should be thoroughly evaluated both qualitatively and quantitatively. Adequate experimental studies, e.g., accuracy, precision, linearity, selectivity, should be accomplished in order to verify the accuracy

of the quantitative results should these results become an integral part of an extractables/leachables correlation. An optimized extraction technique/method can also serve as the basis for development and validation of routine extractables control methods. These fully validated routine extractables control methods can then be used to produce qualitative and quantitative databases of component extractables information which can facilitate correlation of extractables and leachables.

During its model Controlled Extraction Studies, the Working Group chose and optimized extraction techniques/methods for both the sulfur-cured elastomer, one peroxide-cured elastomer, and polypropylene test articles. Extractables were then quantified for the sulfur-cured and polypropylene test articles using the optimized extraction technique/method.

Based on an objective evaluation of all extractables profiles, Soxhlet extraction in methylene chloride was selected for optimization experiments for the sulfur-cured elastomer test article. A timed Soxhlet extraction with a fixed mass of rubber (7 g cut into 20-30 approximately uniform pieces), and with 200 mL of methylene chloride spiked with an internal standard (2-fluorobiphenyl), was performed. The drop rate of methylene chloride in the Soxhlet extractor was approximately 20/min. Samples of methylene chloride extract (1.0 mL taken through the sidearm when boiling stopped) were collected at time intervals of 1, 2, 3, 5, 8, 12 and 16 hours. These samples were then diluted 10:1 with fresh methylene chloride, and analyzed by GC/MS. Selected ion peak area ratios (A_{ion}/A_{is}) were monitored for four of the most significant and representative extractables over the time course. These peak area ratios were then plotted versus extraction time (see Figure 30). Based on the data, it was determined that a 16 hour extraction is suitable.

For the polypropylene test article, reflux extraction with 2-propanol followed by LC/DAD analysis of extracts was chosen for optimization. The levels of the major identified extractables corresponding to the known additives Ultranox 626, Irganox 1010, and Millad 3988 were monitored during the optimization experiment. HPLC conditions were also optimized, including a change in the column and change in ratio of water to acetonitrile for the mobile phase. The new column and mobile phase composition produced better chromatography overall and allowed for only one signal at 200 nm to be used for quantification of Millad 3988. The sample preparation method for extraction and measurement of the analytes in polypropylene was also optimized. This optimization included refinement of the solvent to sample ratio, type of solvent and exposure time.

It was found that the Millad reference standard was not readily soluble in 2-propanol alone, but was soluble in a 50:50 mixture of 2-propanol (IPA) and tetrahydrofuran (THF). The other reference materials were also soluble in this solvent mixture, and the 50/50 IPA/THF solvent was used for both sample extraction and standard preparation. The solvent to sample ratio was evaluated and a ratio of 25 mL solvent to 1 gram of sample with a total surface area of 50 cm^2 appeared to be adequate and was selected for the final method. Extraction time studies were accomplished to determine the optimal length for extraction by 2-propanol reflux. For the example shown in this section, six separate aliquots of polypropylene in 50:50 2-propanol/THF were sampled at six different time intervals. Samples from reflux times of 1, 2, 4, 6, 8, and 10 hours were analyzed under the optimized HPLC conditions. The absolute amounts of each

analyte found for each reference material were calculated at each time interval and plotted versus extraction time.

Figure 30 shows the results of the optimized methylene chloride Soxhlet extraction of the sulfur-cured elastomer test article. Asymptotic levels for all four monitored extractables are clearly reached after approximately 8 hours of extraction time. Figure 31 shows the results of the optimized polypropylene extraction using reflux with 50:50 2-propanol/THF. Asymptotic levels of the target analytes were achieved, with optimal extraction time of about 3 hours. In general, for a first screening, a 24 hour extraction may be an appropriate starting point. However, the timing can be reduced when, as in these cases, it is demonstrated that a shorter extraction time results in asymptotic levels.

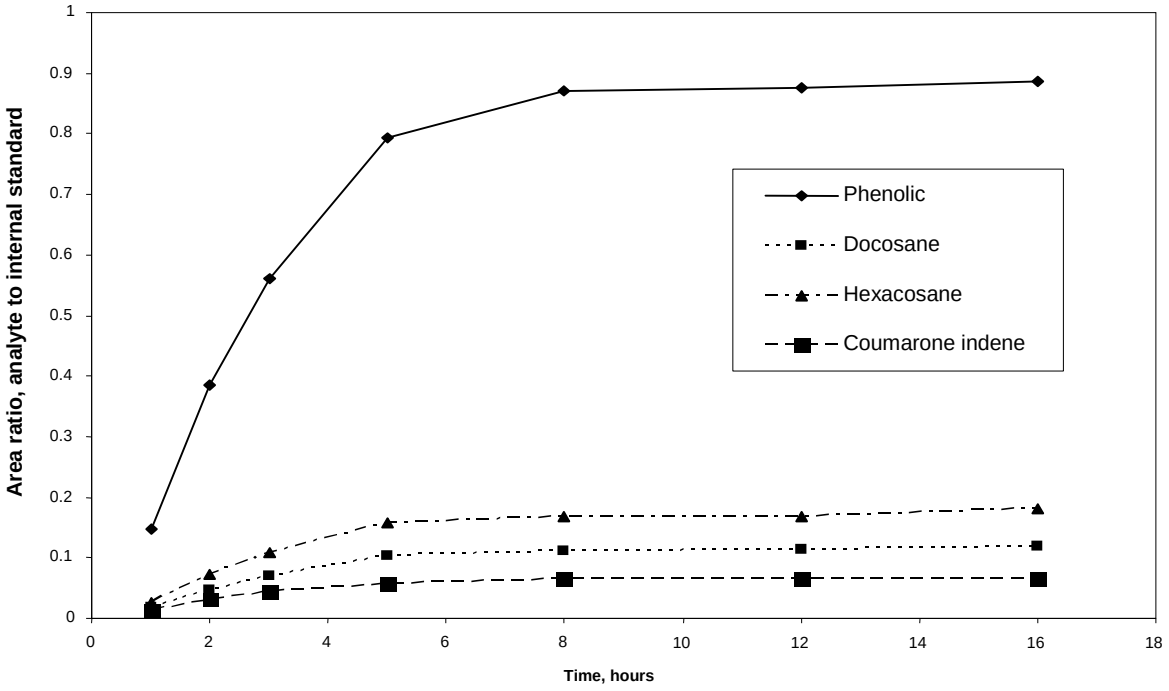


Figure 30. Model extraction optimization experiment (methylene chloride Soxhlet extraction) performed during Controlled Extraction Studies on the sulfur-cured elastomer test article. "Phenolic" = 2,2´-methylene-bis(6-*tert*-butyl-4-ethyl-phenol). "Coumarone indene" = coumarone indene resin identified as a trimer of two indenes with one α-methylstyrene.

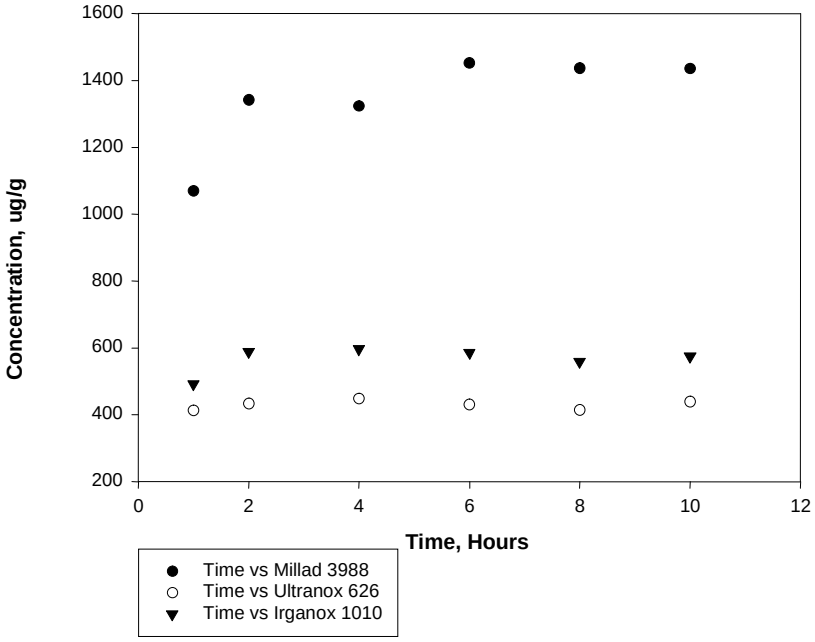


Figure 31. Model extraction optimization experiment (2-propanol reflux extraction) performed during Controlled Extraction Studies on the polypropylene test article.

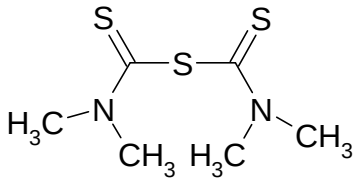
Note that these are but two examples of how extraction technique/method optimization studies might be accomplished during the overall Controlled Extraction Study process. Obviously, there are other study designs that could accomplish the same purpose, and the reader should not infer that the study designs described in this document are the only ones acceptable. However, the end result of extraction technique/method optimization studies should always be the achievement of asymptotic levels of extractables with high overall extractables yields in order to facilitate correlation of extractables and leachables, and to allow for development and validation of appropriate analytical methods for routine control of extractables.

7. Recommendation - Revisit Supplier Information

During the Controlled Extraction Study process, the pharmaceutical development team should compare the qualitative and quantitative extractables profile results with all information on component composition obtained from the component supplier. This comparison is significant because in some cases, Controlled Extraction Studies may detect chemical entities as extractables that are not included in the supplier information. Conversely, supplier information may include additives that are not found in the Controlled Extraction Studies. In the latter case this may mean, among other things, that the extraction and/or analytical methods are not optimal or appropriate for the given test article, or that the particular chemical additive has been consumed in the curing and/or compounding processes for the particular component. In any

case, the absence of a compound or the presence of an unexpected compound in the Controlled Extraction Studies should be investigated.

The Working Group compared results to supplier information throughout its model Controlled Extraction Studies. In the case of the sulfur-cured elastomer test article, it was determined that tetramethylthiuram monosulfide (TMTS) was not detected in any of the extractables profiles, even though this compound was listed as an ingredient in the elastomer formulation. The molecular structure of TMTS is:



TMTS is a vulcanization accelerator and known N-nitrosamine precursor, making it a potential leachable of some interest. An authentic reference standard of TMTS was analyzed by GC/MS under the same analytical conditions used to characterize elastomer extracts. Figure 32 shows a TIC from the GC/MS analysis of authentic TMTS, indicating that this additive would likely be detected in GC/MS profiles of sulfur-cured elastomer extracts. Based on these results it is reasonable to assume that TMTS was significantly consumed during the elastomer polymerization/cross-linking process. However, this result does not relieve the burden of N-nitrosamine testing for this elastomer, as these are themselves reaction products of TMTS and could form during the elastomer curing process.

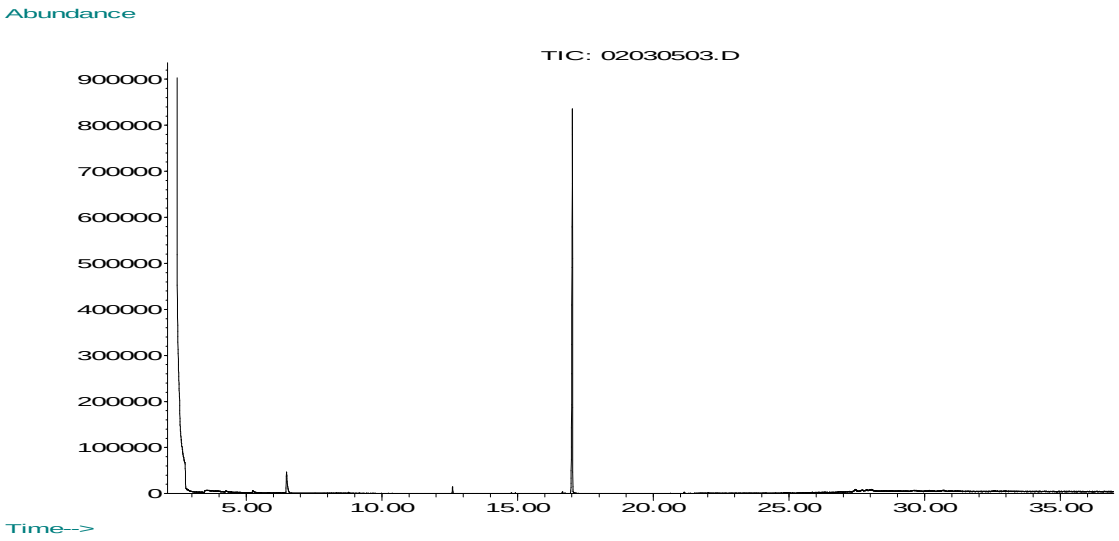


Figure 32. Total Ion Chromatogram from the GC/MS analysis of authentic tetramethylthiuram monosulfide.

The Working Group also encountered an example in which the Controlled Extraction studies identified a compound as an extractable that was not included in the supplier information.

In this case, the phenolic antioxidant Irganox 1076 was detected by GC/MS in extracts from the peroxide-cured elastomer test article for which the Working Group did not have formulation knowledge in advance (see Chapter I, Component Selection). Irganox 1076 was not included in the supplier's ingredients information, provided after conclusion of the study, and it is therefore likely that it was added to the elastomer "base polymer" as an antioxidant. The base polymer is usually synthesized by a different manufacturer than the primary OINDP component supplier, and the component supplier may not have access to additive information for the base polymer.

These examples demonstrate clearly that (i) it is important to obtain component formulation information from the supplier, (ii) this information should be compared to Controlled Extraction Study results, and (iii) that supplier information alone is not adequate to obtain a comprehensive understanding of potential extractables, and therefore leachables, from a given test article.

8. Recommendation - Use of an Analytical Evaluation Threshold

As stated previously, the AET is designed to determine how low one should go in a given extractables profile to identify and evaluate individual extractables. A complete discussion of the AET is presented in Part 3, Chapter IV of this recommendation document.

9. Recommendation - Special Cases

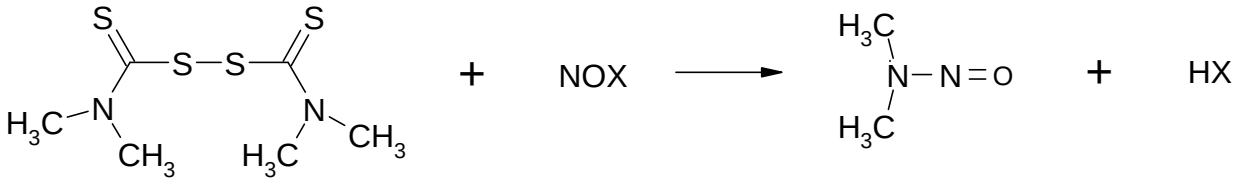
Polycyclic Aromatic Hydrocarbons (PAH's; or Polynuclear Aromatics, PNA's), N-nitrosamines, and 2-mercaptobenzothiozole (MBT) are considered to be "special case" compounds, requiring special characterization studies using specific analytical techniques/methods. Table 4 lists the PNAs and N-nitrosamines which are typically investigated as extractables and leachables in OINDP:

Table 4. PAHs/PNAs and N-nitrosamines Typically Investigated as Extractables and Leachables for OINDP

PAHs/PNAs	N-nitrosamines
Naphthalene	N-nitrosodimethylamine
Acenaphthylene	N-nitrosodiethylamine
Acenaphthene	N-nitrosodi-n-butylamine
Fluorene	N-nitrosomorpholine
Phenanthrene	N-nitrosopiperidine
Anthracene	N-nitrosopyrrolidine
Fluoranthene	
Pyrene	
Benzo(a)anthracene	
Chrysene	
Benzo(b)fluoranthene	
Benzo(k)fluoranthene	
Benzo(e)pyrene	
Benzo(a)pyrene	
Indeno(123-cd)pyrene	
Dibenzo(ah)anthracene	
Benzo(ghi)perylene	

PAHs/PNAs have been associated with carbon black filler used in many types of elastomer, including the sulfur-cured elastomer investigated by the Working Group. Analysis of PAHs/PNAs, either as elastomer extractables or as drug product leachables, usually involves quantitative extraction followed by highly specific and sensitive analysis of resulting extracts. GC/MS with selected-ion-monitoring (SIM)[13] has been reported for analysis of target PAHs/PNAs in Metered Dose Inhaler drug products, for example. Analytical techniques such as GC/MS with SIM are capable of detecting and quantitating PAHs/PNAs at ng/canister levels in MDI drug products and low ppm (part per million) levels in rubber.

N-nitrosamines are reaction products between specific organic precursor molecules, secondary amines (R_2NH) and a "nitrosating agent" (NOX).[14] In the compounding of rubber, secondary amines are likely formed from certain vulcanization accelerators such as thiurams and dithiocarbamates. For example, tetramethylthiuramdisulfide (**I**) can liberate dimethylamine which can then react to form N-nitrosodimethylamine (**II**) as depicted in simplified form below:



I **II**

Potential nitrosating agents include NO^+, N_2O_3, N_2O_4, etc., certain of which can be formed from commonly used chemicals such as sodium nitrite ($NaNO_2$) which has many industrial uses. The formation of N-nitrosamines in rubber has been extensively studied[14-17]. Analysis of N-nitrosamines in rubber as potential extractables is accomplished by quantitative extraction followed by analysis of extracts with Gas Chromatography with Thermal Energy Analysis detection[18,19] (GC/TEA®).[20] GC/TEA is based on the phenomenon of chemiluminescence, a complete discussion of which is beyond the scope of this recommendation document. For a more thorough discussion of N-nitrosamines in rubber and their analysis as extractables, the reader is referred to the previously indicated citations and reference 2. In the experience of the Working Group, analytical methods for N-nitrosamines as leachables in OINDP are usually also based on quantitative extraction and GC/TEA. Sensitivities for N-nitrosamine analytical techniques/methods for rubber are in the low ppb range and low ng/canister range for MDI drug products.

MBT is a known ingredient in the sulfur-cured elastomer. As part of its Controlled Extraction Studies, the Working Group developed a specific and sensitive analytical method for MBT in the sulfur-cured elastomer in order to provide an example of how this special case compound could be investigated.

10. Recommendation – Incorporation of Safety Consultation

Toxicologists should be consulted during the review of extractables profiles obtained during Controlled Extraction Studies so that potential leachables which represent possible safety concerns can be identified and safety evaluated early in the development program. The toxicologist will need to know some identification information and quantitative levels of the individual extractables. Interaction with the toxicologists is discussed further in Appendix 3.

E. Concluding Statement

It cannot be stressed strongly enough that the best practice recommendations for the conduct of Controlled Extraction Studies listed and discussed in this document are not intended to be prescriptive. Scientifically justified alternative approaches to extraction, extract analysis, identification of extractables, special case compound analysis, etc. are not precluded by this recommendation document. However, any OINDP pharmaceutical development team considering significant deviations from any of these recommendations is encouraged to first consult the appropriate regulatory authority.

F. References

1 Norwood, DL; Nagao, L, Lyapustina, S; Munos, M. Application of modern analytical technologies to the identification of extractables and leachables. Am Pharm Rev, **8** (1), pp. 78-87, 2005.

2 Norwood, DL, Paskiet, DM, Granger, AT. Extractables and leachables in drugs and packaging. *Encyclopedia of Pharmaceutical Technology*, 3rd edition, Marcel Dekker, in press, 2006.

3 Jenke, DR. Nomenclature associated with chemical characterization of and compatibility evaluations of medical product delivery systems. PDA J Pharm Sci Technol, **57** (2), pp. 97-108, 2003.

4 McLafferty, FW, Turecek, F. *Interpretation of Mass Spectra*, 4th Edition; University Science Books: Sausalito, California, 1993.

5 Budzikiewicz, H, Djerassi, C., Williams, DH. *Mass Spectrometry of Organic Compounds*; Holden-Day: San Francisco, 1967.

6 Harrison, AG. *Chemical Ionization Mass Spectrometry*; CRC Press: Boca Raton, Florida, 1983.

7 Fenn, JB, Mann, M, Meng, CK, Wong, SF, Whitehouse, CM. Electrospray ionization for mass spectrometry of large biomolocules. Science **246**, pp. 64-71, 1989.

8 Bruins, AP, Covey, TR, Henion, JD. Ion spray interface for combined liquid chromatography/atmospheric pressure ionization mass spectrometry. Anal Chem **59**, pp. 2642-2646, 1987.

9 Busch, KL, Glish, GL, McLuckey, SA. *Mass Spectrometry/Mass Spectrometry: Techniques and Applications of Tandem Mass Spectrometry*; VCH: New York, 1988.

10 Jenke, D. Extractable/leachable substances from plastic materials used as pharmaceutical product containers/devices. J Pharm Sci Technol, **56** (6), pp. 332-364, 2002.

11 Jenke, D. Organic extractables from packaging materials: chromatographic methods used for identification and quantification. J Liq Chromatogr Relat Technol, **26** (15), pp. 2449-2464, 2003.

12 Christman, RF. Guidelines for GC/MS identification. Environ Sci Technol, **16** (3), p. 143A, 1982.

13 Norwood, DL, Prime, D, Downey, BP, Creasey, J, Satinder, SK, Haywood, P. Analysis of polycyclic aromatic hydrocarbons in metered dose inhaler drug formulations by isotope dilution gas chromatography/mass spectrometry. J Pharm Biomed Anal, **13** (3), pp. 293-304, 1995.

14 Willoughby, BG, Scott, KW. *Nitrosamines in Rubber*, Rapra Technology Ltd.: Shawbury, UK, 1997.

15 Spiegelhandler, B, Preussmann, R. Nitrosamines and Rubber. IARC Scientific Publication, **41**, pp. 231-243, 1982,

16 Stevenson, A, Virdi, RS. Nitrogen-free accelerator reduces nitrosamine content of rubber. Elastomerics, **123** (6), pp. 22-29, 1991**.**

17 Layer, RW, Chasar, DW. Minimizing nitrosamines using sterically hindered thiuram disulfides/dithiocarbamates. Rubber ChemTechnol, **67** (2), pp. 299-313, 1994.

18 Gray, JI, Stachiw, MA. Gas chromatographic-thermal energy analysis method for determination of volatile N-nitrosamines in baby bottle rubber nipples: collaborative study. J AOAC, **70** (1), pp. 64-68, 1987.

19 AOAC Official Method 987.05. N-nitrosamines in baby bottle rubber nipples gas chromatographic method first action 1987. AOAC Official Methods of Analysis, Chapter 48, pp. 7-8, 2000.

20 TEA® is a registered trademark of Thermoelectron Corporation.

III. LEACHABLES STUDIES AND ROUTINE EXTRACTABLES TESTING

A. Introduction

In the majority of OINDP pharmaceutical development programs, container closure system component selection and Controlled Extraction Studies are accomplished in series. The two development program phases which follow, drug product leachables studies and "routine" extractables testing of critical components, often proceed in parallel.

A Leachables Study is a laboratory investigation into the qualitative and quantitative nature of a particular OINDP leachables profile(s) over the proposed shelf-life of the product. The purpose of a Leachables Study is to systematically and rationally identify and quantify drug product leachables to the extent practicable, and within certain defined analytical threshold parameters. Leachables Studies typically involve the development and validation of analytical methods capable of detecting and quantifying all potential leachables characterized in the Controlled Extraction Studies, as well as identifying "unspecified" leachables which may have escaped prior characterization or form via chemical reaction in the drug product formulation matrix. Leachables Studies are most often accomplished as part of a larger drug product stability program on multiple batches of drug product, using multiple component batches, stored under a variety of conditions through the intended shelf-life of the product, designed to support registration activities. Since these large drug product stability studies involve analysis of samples at multiple time-points, it is possible to discern trends in drug product leachables profiles over time and storage condition. Like the Controlled Extraction Study, the Leachables Study can be framed as a Trace Organic Analysis problem, with the sample matrix being the drug product formulation. Analytical methods for leachables analysis must quantitatively recover leachables from the drug product matrix, separate and individually detect them with appropriate sensitivity. Analytical techniques most often employed for Leachables Studies are the same as those used in Controlled Extraction Studies, namely GC/MS, LC/MS and LC/UV (or LC/DAD). Leachables Studies provide information in support of developing an extractables/leachables correlation, and for the establishment of drug product leachables specifications and acceptance criteria.

Routine Extractables Testing is the process by which OINDP container closure system critical components are qualitatively and quantitatively profiled for extractables, either for purposes of establishing extractables acceptance criteria, or release according to already established acceptance criteria. Like the analytical methods used in Leachables Studies, those used for Routine Extractables Testing must be capable of detecting and quantifying all extractables characterized in the Controlled Extraction Studies, as well as identifying "unspecified" extractables which could result from unanticipated changes in critical component ingredients or some external contamination. However, Routine Extractables Testing analytical methods must also be highly rugged and robust, making them easily transferable and useful in quality control and manufacturing environments. As a result of these requirements, it is common practice to employ analytical techniques which lend themselves to methods with the desired characteristics. For example, when GC/MS was used for Controlled Extraction Studies, a Routine Extractables Testing method could be based on the more rugged and robust GC/FID (Gas Chromatography/Flame Ionization Detection). LC/MS Controlled Extraction Study methods could be converted to LC/UV Routine Extractables Testing methods, as long as the UV

detector is sufficiently sensitive for the extractable under consideration. Again, like Leachables Study analytical methods, analytical methods used for Routine Extractables Testing must be validated according to accepted industry practice. Early in the OINDP development process, Routine Extractables Testing is used to create a qualitative/quantitative extractables database which can be used to help establish an extractables/leachables correlation and to develop critical component extractables specifications and acceptance criteria. Later in the development process and post-approval, Routine Extractables Testing is used to release critical components for drug product manufacture according to previously established acceptance criteria. The pharmaceutical development processes outlined in these recommendations including the safety qualification decision tree, can be applied to evaluation necessary due to post approval changes.

This chapter lists and elaborates the Working Group's best practice recommendations for Leachables Studies and Routine Extractables Testing in OINDP pharmaceutical development programs. Data and information developed by the Working Group in the conduct of its laboratory investigations, including Controlled Extraction Studies and simulated Leachables Studies, are used, where appropriate, to illustrate individual recommendations.

B. Scope and Application for Leachables Studies and Routine Extractables Testing

The scope and application of drug product Leachables Studies is discussed in some detail in the following chapter of this recommendation document (Part 3, Chapter IV), which deals with the leachables/extractables Analytical Evaluation Threshold (AET). To summarize:

1. ***Comprehensive Leachables Studies should always be accomplished for Metered Dose Inhaler (MDI) drug products, and should generally be accomplished for Nasal Spray and Inhalation Spray drug products. If scientifically justified, Leachables Studies may not need to be accomplished for particular Nasal Spray or Inhalation Spray drug products.***

2. ***Leachables Studies (either stability studies or "one-time" characterization studies) are required for the to be marketed Dry Powder Inhaler (DPI) drug products only if potential leachables, i.e., extractables, of safety concern are identified in the Controlled Extraction Studies (see Chapter II for appropriate recommendations) at or above the AET level from the unit dose container closure system and other critical components of the device which may have continuous long term contact with the drug product formulation.***

3. ***For Inhalation Solution and Suspension drug products, Leachables Studies are not required if it can be scientifically demonstrated that:***

    a. ***Aqueous and/or drug product formulation extracts of Inhalation Solution direct formulation contact container closure system materials yield no extractables, under appropriate stress conditions, at Final AET levels, or no extractables above final AET levels with safety concern; AND***

    b. ***There is no evidence for migration of organic chemical entities through the unit dose container into the drug product formulation.***

Leachables Studies should have the following goals:

1. *To help establish an extractables/leachables correlation.*

2. *To understand the trends in drug product leachables levels over the shelf-life of the product.*

3. *To determine maximum leachables levels up to the proposed end of shelf-life of the product.*

4. *To support a comprehensive safety evaluation of drug product leachables.*

5. *To establish drug product leachables specifications and acceptance criteria, should these be required.*

Routine Extractables Testing is performed on all critical components of OINDP container closure systems. Routine Extractables Testing has the following general goals:

1. *To establish extractables specifications and acceptance criteria for OINDP critical container closure system components.*

2. *To help ensure that the leachable profile in the drug product is maintained within appropriate limits.*

3. *To release OINDP container closure system critical components according to established specifications and acceptance criteria, which are designed to:*

    a. *Control the identities and levels of extractables identified during Controlled Extraction Studies; and*

    b. *Detect "unspecified" extractables which could be present as the result of component ingredient changes, manufacturing changes, external contamination, or other causes.*

Acceptance criteria for OINDP critical component extractables should include the following:

1. *Confirmation of extractables identified in Controlled Extraction Studies.*

2. *Quantitative limits for extractables identified in Controlled Extraction Studies.*

3. *Quantitative limits for unspecified extractables.*

The actual form and statement of extractables specifications and acceptance criteria depend on many factors, including the risk associated with detecting drug product leachables associated with individual critical components. In DPI non-contact critical components, for example, there is no risk of detecting associated leachables and the level of extractables control required would not be the same as for an MDI valve critical component where the risk of

detecting associated leachables is very high. Additional recommendations as to the form and statement of extractables specifications and acceptance criteria are beyond the scope of this PQRI project, and are left to the OINDP pharmaceutical development team in consultation with regulatory authorities.

C. Recommendations for Leachables Studies and Routine Extractables Testing

1. ***Analytical methods for the qualitative and quantitative evaluation of leachables should be based on analytical technique(s)/method(s) used in the Controlled Extraction Studies.*** During the conduct of comprehensive Controlled Extraction Studies, analytical techniques and methods would have been developed and applied to critical OINDP container closure system components in order to develop a complete understanding of potential leachables. Given that one of the principal goals of Leachables Studies is to allow for an extractables/leachables correlation, it is logical and appropriate for the analytical methods used in such Leachables Studies to be based on those used for the Controlled Extraction Studies. For example, if a GC/MS method was developed and optimized for characterizing an elastomeric component's extractables profile, a similar method based on GC/MS should be developed and applied to the corresponding drug product leachables profile. The leachables method should be developed so as to optimize the recovery of potential leachables from the drug product matrix, as well as being validated according to common pharmaceutical industry practice.

2. ***Leachables Studies should be guided by an Analytical Evaluation Threshold (AET) that is based on an accepted safety concern threshold.*** The AET is designed to determine how low one should go in a given leachables profile to identify, quantify and evaluate individual leachables. A complete discussion of the AET is presented in Part 3, Chapter IV of this document.

3. ***A comprehensive correlation between extractables and leachables profiles should be established***. A qualitative correlation can be established if all leachables detected can be qualitatively linked directly or indirectly to an extractable. A quantitative correlation can be established if the levels of individual leachables determined at the end of drug product shelf-life are less than or equal to the levels of corresponding extractables. Both qualitative and quantitative correlations should include multiple batches of components and multiple batches of drug product (including multiple stability time-points, stability storage conditions and drug product orientations). For example, to establish correlations, the same batches of components used in Controlled Extraction Studies should be used, if possible, in the drug product batches that are tested for leachables. Extraction conditions should achieve approximately asymptotic levels of extractables, if possible. Leachable data should be acquired through the intended shelf-life of the product. It is further recommended that the sponsor again revisit available supplier information to ensure that all known critical component ingredients are accounted for.

4. ***Specifications and acceptance criteria should be established for leachables profiles in OINDP.*** For any OINDP in which leachables studies are required (such as for MDIs, Nasal Sprays and Inhalation Sprays, and for DPIs and Inhalation Solutions in certain cases) the development of specifications and acceptance criteria for leachables profiles are recommended by the Working Group. The implementation of leachables testing for any particular OINDP is a policy decision which should be negotiated between a sponsor and the appropriate regulatory authority. However, if qualitative and quantitative extractables/leachables correlations (as defined in recommendation 3, above) are established, the Working Group suggests that leachables specifications and acceptance criteria should be noted as ***"if tested will comply"***. In this case, leachables would be controlled indirectly through routine control of critical component extractables profiles.

5. ***Analytical methods for Routine Extractables Testing should be based on the analytical technique(s)/method(s) used in the Controlled Extraction Studies.*** As previously stated, it is common practice to use Routine Extractables Testing methods to assist in the development of extractables/leachables correlations. Given this, it is again both logical and appropriate to develop Routine Extractables Testing methods based on the analytical techniques and methods used for the Controlled Extraction Studies. Remember, however, that "based on" does not mean "identical to" and again as previously stated, Routine Extractables Testing analytical methods have requirements for ruggedness and robustness that are greater than those for Controlled Extraction Study methods. Therefore, it is appropriate and acceptable to use GC/FID methods which are based on GC/MS methods, and LC/UV methods (validated to insure appropriate detection sensitivity) which are based on LC/MS methods.

6. ***Routine Extractables Testing should be performed on critical components using appropriate specifications and acceptance criteria.*** The Working Group recommends that extractables profiles from OINDP container closure system critical components be routinely monitored for extractables based on established specifications and acceptance criteria. As stated in Recommendation 4, such testing may obviate the need to implement routine testing of drug product leachables.

7. ***Analytical methods for Leachables Studies and Routine Extractables Testing should be fully validated according to accepted parameters and criteria.*** Any analytical method developed either for release of OINDP critical components based on extractables profiles, or for testing of leachables over the shelf-life of a drug product, should be fully validated according to accepted pharmaceutical industry practice and the highest scientific standards.

8. ***Polycyclic Aromatic Hydrocarbons (PAH's; or Polynuclear Aromatics, PNA's), N-nitrosamines, and 2-mercaptobenzothiazole (MBT) are considered to be "special case" compounds, requiring evaluation by specific analytical techniques and technology defined thresholds for Leachables Studies and***

Routine Extractables Testing. These particular compound classes and chemical entities have historically demanded greater scrutiny and are therefore considered separately from other extractables and leachables.

9. ***Qualitative and quantitative leachables profiles should be discussed with and reviewed by pharmaceutical development team toxicologists so that any potential safety concerns regarding individual leachables are identified as early as possible in the pharmaceutical development process.*** Information from Leachables Studies will allow pharmaceutical development team toxicologists to assess potential patient exposure to individual organic leachables and to understand and evaluate potential safety concerns.

D. Discussions and Illustrative Data for Leachables Studies and Routine Extractables Testing Recommendations

In order to assist in the development of its recommendations and to provide illustrative data, the Working Group conducted a "simulated" Metered Dose Inhaler (MDI) leachables study. In this study, quantities of sulfur-cured elastomer were placed in glass formulation bottles filled with CFC 11 (trichlorofluoromethane), and stored under accelerated conditions in a stability chamber.

Note: Simulated leachables studies are NOT recommended for leachables testing in an actual pharmaceutical development process, and such recommendations should not be inferred from this document. Such simulated studies should not be used as a substitute for comprehensive Controlled Extraction Studies, or for comprehensive Leachables Stability Studies where these are required.

1. Recommendation -- Analytical Methods for Leachables

The recommendation that analytical methods for the qualitative and quantitative evaluation of leachables should be based on the analytical technique(s)/method(s) used in the corresponding Controlled Extraction Studies, is illustrated by GC/MS Total Ion Chromatograms presented in Figures 1-3. Figure 1 shows an extractables profile, i.e., GC/MS TIC, from the sulfur-cured elastomer (24 hour Soxhlet extraction in methylene chloride) acquired during the Controlled Extraction Study phase of the Working Group's laboratory investigations. Figure 2 shows a similar extractables profile (16 hour Soxhlet extraction in methylene chloride), acquired during the extraction optimization phase of the work, with internal standard (2-fluorobiphenyl) added. Figure 3 shows a leachables profile acquired with an optimized sample preparation procedure and identical GC/MS conditions (1 week storage at 40°C, 75% relative humidity).

As stated above, one of the principal goals of a Leachables Study is to establish an extractables/leachables correlation. Examination and comparison of these extractables and leachables GC/MS profiles clearly suggests, given optimized extraction procedures for the elastomer and fully optimized and validated leachables methods, that both qualitative and quantitative correlations of extractables and leachables are possible. Visual inspection of the chromatogrms clearly indicates that the leachables and extractables profiles are qualitatively identical. This observation was confirmed by careful evaluation of the GC/MS data, including

evaluation of appropriate control samples from the leachables study. Differences between the analytical techniques/methods used in the Controlled Extraction Studies and Leachables Studies would only serve to complicate the establishment of an extractables/leachables correlation.

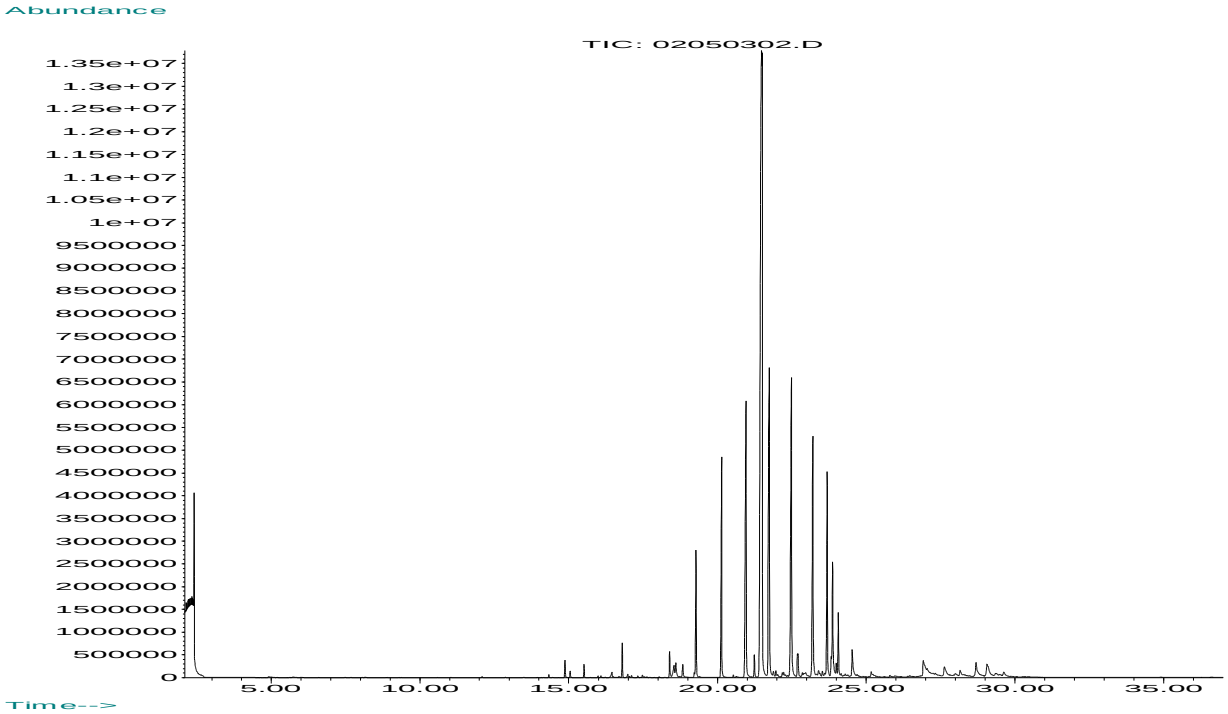


Figure 1. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the sulfur-cured elastomer test article, 24 hour methylene chloride Soxhlet extraction.

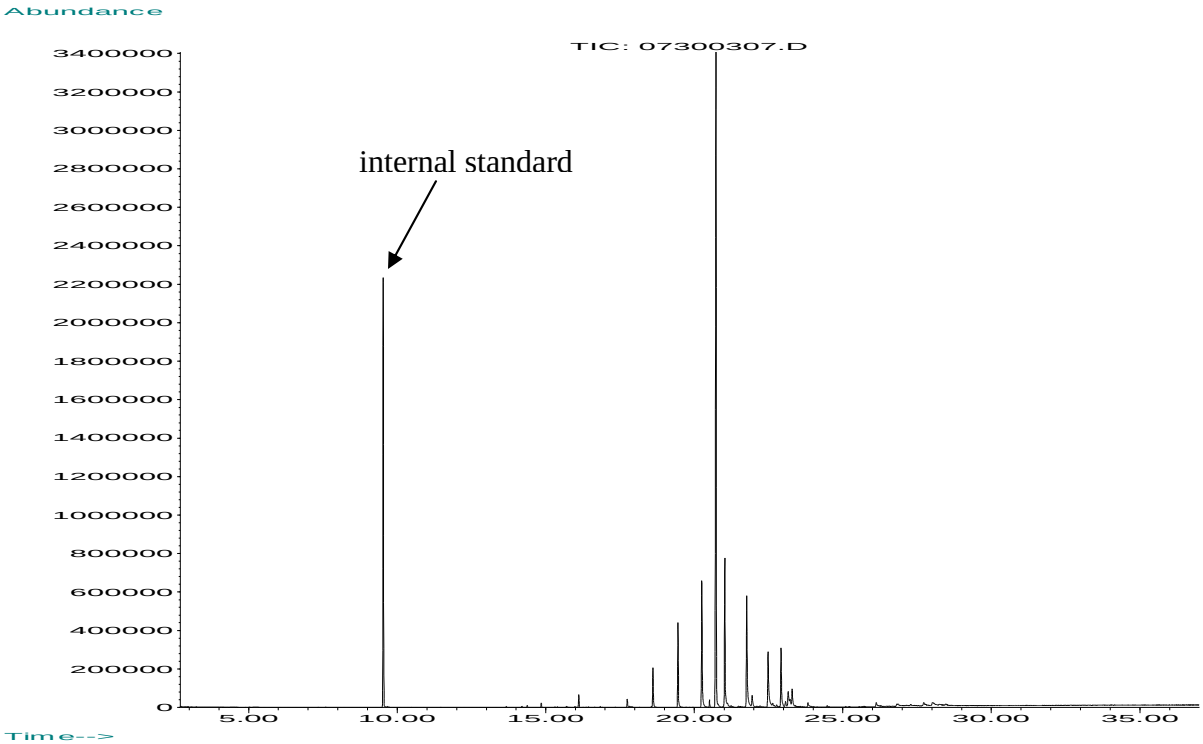


Figure 2. GC/MS (Gas Chromatography/Mass Spectrometry) extractables profile (Total Ion Chromatogram, TIC) of the sulfur-cured elastomer test article, 16 hour methylene chloride Soxhlet extraction.

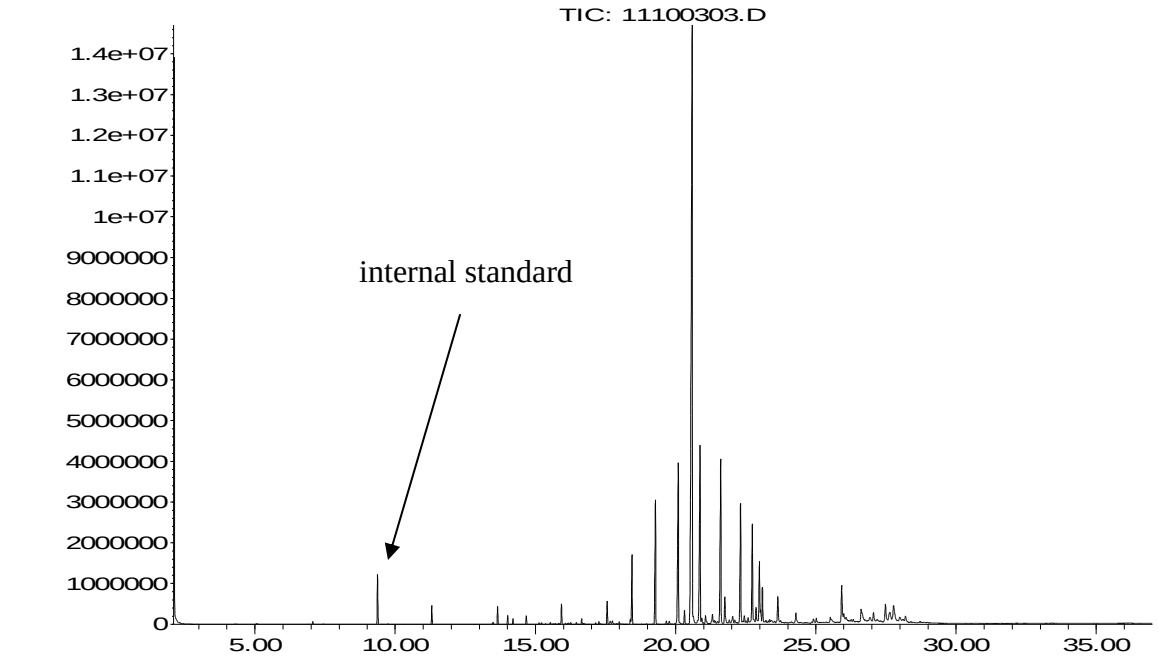


Figure 3. GC/MS (Gas Chromatography/Mass Spectrometry) leachables profile (Total Ion Chromatogram, TIC) of the sulfur-cured elastomer test article, 1 week storage at 40°C and 75% relative humidity.

2. Recommendation – Use of the AET

See Part 3, Chapter IV for a complete discussion of the Analytical Evaluation Threshold (AET) concept.

3. Recommendation -- Establishing a Leachables/Extractables Correlation

The significance of a ***correlation*** between extractables and leachables profiles cannot be overstated. A correlation should be both qualitative and quantitative, and should be demonstrable over multiple batches of drug product to end of shelf-life, and multiple batches of container closure system critical components.

(a) Definitions of qualitative and quantitative correlation

Qualitative Correlation: A *qualitative correlation* can be established if all compounds detected in validated leachables studies can be linked qualitatively either directly or indirectly to an extractable identified in comprehensive Controlled Extraction Studies or during Routine Extractables Testing. A direct qualitative correlation is relatively simple, for example:

I. Stearic acid is a known ingredient in a particular MDI dose metering valve critical component, i.e., as technical grade Calcium Stearate.

II. Stearic acid is *confirmed* by GC/MS in methylene chloride Soxhlet extracts of the critical component in question during Controlled Extractions Studies. Stearic acid is also confirmed in 30 batches of the critical component during Routine Extractables Testing with a validated GC/FID method.

III. Stearic acid is *confirmed* by a validated GC/MS method to be present in definitive registration batches of drug product, at various time-points over the proposed shelf-life of the product, under different storage conditions, and different product orientations.

An indirect qualitative correlation is only slightly more challenging:

I. Stearic acid is a known ingredient in a particular MDI dose metering valve critical component, i.e., as technical grade Calcium Stearate.

II. Stearic acid is *confirmed* by GC/MS in methylene chloride Soxhlet extracts of the critical component in question during Controlled Extractions Studies. Stearic acid is also confirmed in 30 batches of the critical component during Routine Extractables Testing with a validated GC/FID method.

III. Ethyl stearate is *confirmed* by a validated GC/MS method to be present in definitive registration batches of drug product, at various time-points over the proposed shelf-life of the product, under different storage conditions, and different product orientations.

IV. The MDI drug product formulation is known to contain 10% ethanol which can react with stearic acid to form ethyl stearate.

Qualitative correlations obviously require some knowledge and understanding of the chemistry and reactivity of extractables and chemical additives to rubber and plastic. It is important to be aware that many of these chemical additives, such as polymerization agents, accelerators, antioxidants, stabilizers etc, are by their very nature reactive species.

Note that one does not need to have *confirmed* identifications of particular leachables and extractables in order to establish a qualitative correlation. Information available from analytical techniques such as GC/MS and LC/MS allow for leachables/extractables qualitative correlations of chemical entities with *confident* and *tentative* identifications. *Confirmed* and *confident* levels of identification are generally required for toxicologic evaluation of leachables.

Quantitative Correlation: A *quantitative correlation* between a leachable and an extractable can be made if the level of the leachable is demonstrated to be consistently less than that of the extractable(s) to which it is qualitatively correlated. For an individual batch of OINDP, this quantitative correlation should be valid through the proposed end of shelf-life, and across all accelerated storage conditions and product orientations. Quantitative correlations are best accomplished using data from a significant number of critical component batches, acquired using validated Routine Extractables Testing analytical methods. For example:

I. Stearic acid is shown to have a qualitative leachables/extractables correlation (as defined above) in an MDI drug product.

II. Comprehensive Leachables Studies show stearic acid to have a maximum level in drug product of 50 µg/canister; across all definitive registration batches of drug product, stability storage conditions, drug product orientations, and stability time-points to the proposed end of shelf-life.

III. A database of 50 critical component batches analyzed by a validated Routine Extractables Testing analytical method quantitates stearic acid at 800 µg/g ±100 (standard deviation, i.e., 12.5% relative standard deviation).

IV. Given that there is one 150 mg critical component per MDI valve, the anticipated maximum level of stearic acid as a drug product leachable would be 120 ±15 µg/canister. This result represents a positive quantitative correlation.

(b) **Additional points regarding leachables/extractables correlation**

In establishing both qualitative and quantitative leachables/extractables correlations it is highly recommended that the pharmaceutical team compare:

- Leachables profiles from multiple (at least 3) drug product definitive registration batches using specific batches of critical components, with qualitative and quantitative extractables profiles of those **specific component batches**. For example, the leachables profiles from MDI registration batches should be

compared with the extractables profiles of the components that make up the valves used in those registration batches.

- Leachables profiles from multiple drug product registration batches with extractables profiles from multiple batches of critical components (which may not have been used in the drug product registration batches). This comparison is intended to check the consistency of correlations between extractables profiles from multiple component batches and leachables profiles from multiple drug product batches.

- If a qualitative and quantitative correlation cannot be established, the source of the problem should be determined and corrected. Potential sources include excessive variability in component composition and/or manufacturing processes, changes in drug product formulation, inadequate Controlled Extraction Studies, and inappropriate or poorly validated leachables and extractables methods.

4. Recommendation -- Specifications and Acceptance Criteria for Leachables

Leachables specifications should include a fully validated analytical test method. The acceptance criteria for leachables should apply over the proposed shelf-life of the drug product, and should include:

1. *Quantitative limits for known drug product leachables monitored during product registration stability studies.*

2. *A quantitative limit for "new" or "unspecified" leachables not detected or monitored during product registration stability studies.*

Quantitative acceptance criteria should be based on leachables levels, and trends in leachables levels, observed over time and across various storage conditions and drug product orientations during product registration stability studies, with the application of appropriate statistical analysis. A comprehensive correlation, as defined and elaborated above, may obviate the need for routine implementation of drug product leachables specifications and acceptance criteria. This, of course, further assumes:

1. Adequate information from critical component suppliers (as defined in Chapter I), with an adequate evaluation of this information.

2. Complete understanding and control of critical component fabrication and manufacturing processes.

3. Adequate and comprehensive Controlled Extraction Studies on all critical components.

4. Validated leachables analytical methods and a comprehensive Leachables Study.

5. Validated Routine Extractables Testing analytical methods and an adequate database of critical component extractables profiles.

6. Appropriate specifications and acceptance criteria for extractables from critical components.

The Working Group emphasizes that the requirement for establishment and implementation of leachables specifications and acceptance criteria for any particular OINDP is a regulatory policy matter, and therefore considered to be outside the scope of the Working Group's consideration.

5. Recommendation -- Analytical Methods for Routine Extractables Testing

Extractables/leachables correlations are best established using the results of comprehensive Controlled Extraction Studies, and databases of critical component extractables profiles acquired with fully optimized and validated extractables analytical methods. It is, therefore, often the case that Routine Extractables Testing analytical methods are employed to create such databases of extractables profiles. As stated previously, it is both logical and appropriate to develop Routine Extractables Testing methods based on the analytical techniques and methods used for the Controlled Extraction Studies. Remember, however, that "based on" does not mean "identical to" and again as previously stated, Routine Extractables Testing analytical methods have requirements for ruggedness and robustness that are greater than those for Controlled Extraction Study methods. Therefore, it is appropriate and acceptable to use GC/FID methods which are based on GC/MS methods, and LC/UV methods which are based on LC/MS methods.

Consider the GC/FID extractables profile of the sulfur-cured elastomer shown in Figure 4 (24 hour Soxhlet extraction in methylene chloride), and compare with the GC/MS extractables profiles in Figures 1 and 2. Visual inspection clearly suggests that the GC/MS and GC/FID extractables profiles are qualitatively similar, and this was confirmed by careful evaluation of the data, and validation of the GC/FID method. In a quality control or manufacturing environment, the greater ruggedness and robustness of GC/FID is a significant advantage. Further, the relative costs of instrumentation and the relative requirements for training and expertise of laboratory staff, also suggest advantages of GC/FID over GC/MS. These statements are also true for LC/UV methods as compared with LC/MS methods (perhaps more so).

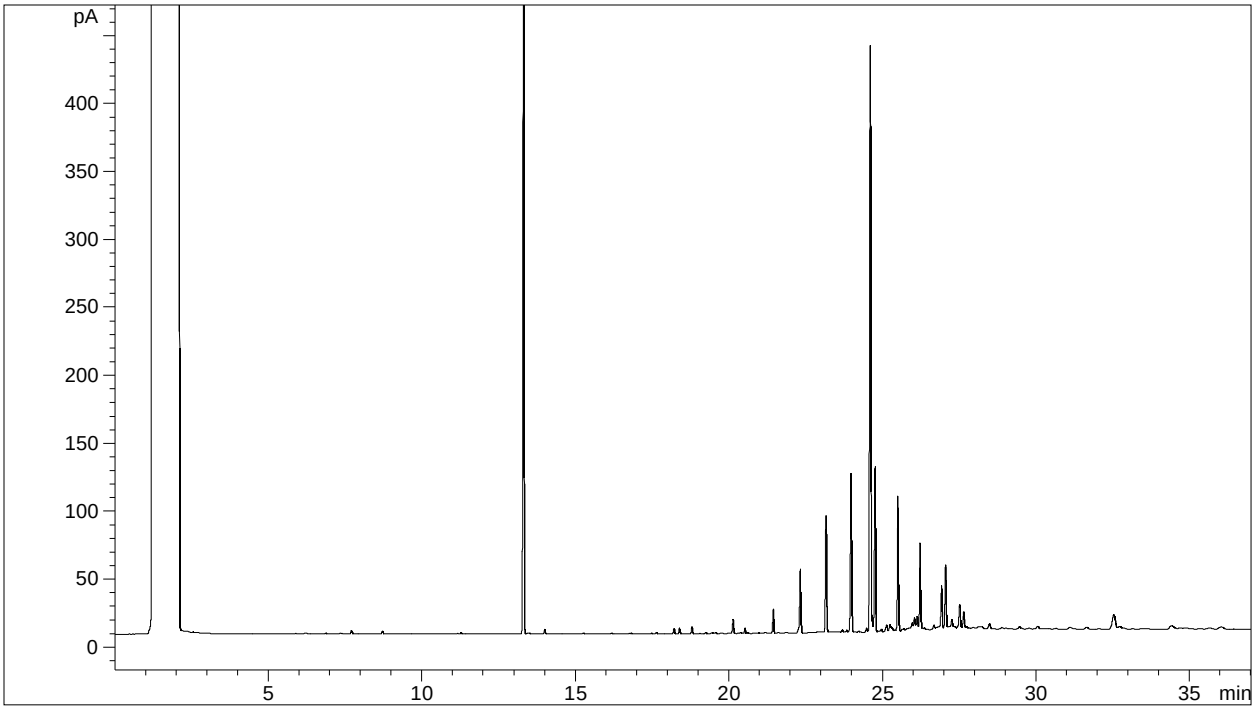


Figure 4. GC/FID (Gas Chromatography/Flame Ionization Detection) extractables profile of the sulfur-cured elastomer test article, 24 hour methylene chloride Soxhlet extraction.

6. Recommendation -- Routine Extractables Testing on all Critical Component Batches

Routine Extractables Testing should be performed on OINDP critical components prior to drug product manufacture. Critical components should be released to drug product manufacture based on carefully defined specifications and acceptance criteria established through:

1. A complete understanding of critical component composition(s), ingredients, and compounding/fabrication processes.

2. Comprehensive Controlled Extraction Studies.

3. A significant database of extractables profiles obtained with fully optimized and validated Routine Extractables Testing analytical methods.

4. A complete leachables/extractables correlation.

The actual form and statement of specifications and acceptance criteria will depend on factors such as the type of OINDP, the type of critical component (such as contact or non-contact with the drug product formulation), adequacy of leachables/extractables correlation, etc.

Acceptance criteria for OINDP critical component extractables can include the following:

1. *Confirmation of extractables identified in Controlled Extraction Studies.*
2. *Quantitative limits for extractables identified in Controlled Extraction Studies.*
3. *A quantitative limit for "new" or " unspecified" extractables not detected during Controlled Extraction Studies.*

The Working Group recognizes that there are many possible ways for setting acceptance criteria, and does not recommend any particular approach to establishing such criteria. For example, quantitative limits need not necessarily be established for all extractables identified in Controlled Extraction Studies, but could be established for major extractables representative of major chemical additives in the component formulation.

Failure of a particular batch of critical component to meet established acceptance criteria suggests either an unapproved change in critical component ingredients, or an unapproved change (or problem) with critical component compounding/fabrication processes. In order to prevent critical component extractables profile failures, and to ensure that critical component quality is maintained, it is important that the sponsor work closely with component suppliers to control critical component compounding/fabrication processes. The sponsor should also clarify to the supplier the sponsor's expectations regarding changes to component ingredients, compounding, fabrication, or other manufacturing processes, including prior notification of such changes.

It is recommended by the Working Group that sponsors develop procedures for investigating Routine Extractables Testing acceptance criteria failures, i.e., Out of Specification, or OOS, procedures. Further, the Working Group recommends that sponsors monitor all critical component extractables profiles for qualitative or quantitative changes which are within established acceptance criteria, and develop procedures for investigating and understanding the root causes of such changes. Careful monitoring of critical component extractables profiles will likely result in fewer failures and OOS investigations.

7. Recommendation – Validation of Analytical Methods for Leachables Studies and Routine Extractables Testing

As previously stated, any analytical method developed either for release of OINDP critical components based on extractables profiles, or for testing of leachables over the shelf-life of a drug product, should be fully validated according to accepted pharmaceutical industry practice and the highest scientific standards. The following documents are referenced:

1. ICH Harmonized Tripartite Guideline, Text on Validation of Analytical Procedures Q2A, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.

2. ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Methodology Q2B, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.

3. Reviewer Guidance – Validation of Chromatographic Methods, Center for Drug Evaluation and Research (CDER), United States Food and Drug Administration, November, 1994.

4. Draft Guidance for Industry – Analytical Procedures and Methods Validation – Chemistry, Manufacturing, and Controls Documentation, Center for Drug Evaluation and Research (CDER), United States Food and Drug Administration, August, 2000.

5. Michael E. Swartz and Ira S. Krull, *Analytical Method Development and Validation*, Marcel Dekker, Inc., New York, 1997.

The Working Group accomplished limited method validation exercises for:

- A methylene chloride Soxhlet extraction/GC-FID analytical test method for the sulfur-cured elastomer test article.

- A 2-propanol reflux extraction/HPLC-UV analytical test method for the polypropylene test article.

These methods would, in principal, be suitable as Routine Extractables Testing methods for these materials. Following is a summary of recommendations based on the laboratory investigations and the experiences of the Working Group:

(a) Development and Validation of Leachables Analytical Methods

Analytical techniques and procedures for the quantitative recovery of leachables from drug product formulation matrices are a function of the type of drug product, e.g., MDI, Inhalation Solution. For example, leachables in an MDI suspension drug product with CFC propellant could be recovered for GC/MS or GC/FID analysis by filtering the suspended drug substance particles from the cooled formulation and capturing the resulting filtrate in a solvent suitable for GC analysis, e.g., methylene chloride. Leachables in an aqueous based Inhalation Solution drug product could be quantitatively recovered by methylene chloride liquid-liquid extraction, with subsequent analysis of the extract by GC/MS or GC/FID. During the method development phase of the overall exercise, the following should be accomplished:

- An "extraction" procedure should be developed which is designed and optimized for the recovery of potential leachables from the drug product matrix. One way to approach this is to use authentic reference compounds from *confirmed* extractables identifications accomplished during the Controlled Extraction Studies. These reference compounds should at least represent the major extractables, i.e., potential leachables, observed, as well as represent known ingredients and additives in appropriate critical components. Recovery of reference compounds could be optimized by spiking into a drug product formulation matrix.

- The linear dynamic range of the analytical method should be established based on levels of potential leachables anticipated from quantitative Controlled Extraction Studies and Routine Extractables Testing of critical components.

- The Limit of Quantitation of the method should be established with consideration of the appropriate AET.

For method validation:

- The method should be validated according to the ICH validation characteristics of a quantitative impurity test. These validation characteristics include: Accuracy, Precision (Repeatability, Intermediate Precision), Specificity, Limit of Quantitation (LOQ), Linearity, and Range. In addition, System Suitability parameters should be established and a Robustness evaluation should be accomplished. For further detailed discussion see the references cited above.

  Note that in certain cases it may be appropriate to validate leachables methods as "Limit Tests", in which case only Specificity and Limit of Detection (LOD) need be considered.

- Accuracy can be determined through the analysis of spiked samples. The spiking matrix could be an actual drug product, in which case a standard additions experiment would be required since the drug product spiking matrix would contain an endogenous level of leachables, or a spiking matrix created in the laboratory from the known drug product formulation ingredients. Spiking levels should be chosen so as to be representative of anticipated leachables levels based on results from quantitative Controlled Extraction Studies and Routine Extractables Testing.

(b) Development and Validation of Routine Extractables Testing Analytical Methods

Extraction procedures for critical components should be based on the optimized procedures from the quantitative Controlled Extraction Studies, and should be demonstrated to show asymptotic levels of extractables. If the Controlled Extraction Study extraction procedure is to be directly transferred to routine extractables testing application, then the results of any method optimization and verification experiments could be directly applied. Further:

- The linear dynamic range of the analytical method should be established based on levels of extractables anticipated from quantitative Controlled Extraction Studies of critical components.

- The Limit of Quantitation of the method should be established with consideration of the appropriate AET.

For method validation:

- The method should be validated according to the ICH validation characteristics of a quantitative impurity test. These validation characteristics include: Accuracy,

Precision (Repeatability, Intermediate Precision), Specificity, Limit of Quantitation (LOQ), Linearity, and Range. In addition, System Suitability parameters should be established and a Robustness evaluation should be accomplished. For further detailed discussion see the references sited above.

Note that in certain cases it may be appropriate to validate routine extractables methods as "Limit Tests", in which case only Specificity and Limit of Detection (LOD) need be considered.

- Accuracy can be determined through the analysis of spiked samples. The spiking matrix could be an extract taken through the extraction procedure minus the component sample. Spiking levels should be chosen so as to be representative of anticipated extractables levels based on results from quantitative Controlled Extraction Studies.

Note: Validation parameter acceptance criteria should be determined for each individual leachables and routine extractables testing analytical method. The results obtained by the Working Group are only applicable to those particular analytical methods which the Working Group evaluated, and should not be used to establish validation acceptance criteria for any sponsor analytical methods.

8. Recommendation - Special Cases

Polycyclic Aromatic Hydrocarbons (PAH's; or Polynuclear Aromatics, PNA's), N-nitrosamines, and 2-mercaptobenzothiazole (MBT) have historically demanded greater scrutiny and are therefore considered separately from other extractables and leachables. For additional details, the reader is referred to the discussion in Chapter II of this document.

In certain cases, such as for MDI valve elastomeric components and MDI drug products, the establishment and implementation of leachables and/or extractables specifications and acceptance criteria may be required for special cases as a matter of regulatory policy. Should this be the case, fully optimized and validated analytical test methods should be available for implementation. For validation, leachables and extractables methods for special cases should be treated as other leachables/routine extractables testing methods.

9. Recommendation – Incorporation of Safety Consultation

Information from Leachables Studies will allow pharmaceutical development team toxicologists to assess potential patient exposure to individual organic leachables and to understand and evaluate potential safety concerns. Interaction with the toxicologists is discussed further in Appendix 3.

IV. THE ANALYTICAL EVALUATION THRESHOLD (AET)

A. Introduction

The *Analytical Evaluation Threshold (AET)* concept proposed by the Working Group and described in this section, acts as a critical guide for any OINDP pharmaceutical development team in its analytical characterization of leachables and extractables. The AET for an individual OINDP is derived directly from the *Safety Concern Threshold (SCT)*, which is defined in terms of absolute exposure of a patient to any individual organic leachable contained in an OINDP. The SCT proposed by the Working Group is:

0.15 µg/day for an individual organic leachable

The SCT represents the threshold below which a leachable would have a dose so low as to present negligible safety concerns from carcinogenic and noncarcinogenic toxic effects.

It is again important to point out that Polycyclic Aromatic Hydrocarbons (PAH's; or Polynuclear Aromatics, PNA's), N-nitrosamines, and 2-mercaptobenzothiazole (MBT) are considered to be "special case" compounds, requiring evaluation by specific analytical techniques and technology defined thresholds. These "special case" compounds are not to be evaluated as either OINDP leachables or extractables using the AET concept proposed in this section.

The challenge for the pharmaceutical development team is to convert the ***absolute*** SCT into an analytically useful threshold defined in terms ***relative*** to the parameters of a particular OINDP. The proposed AET represents such an analytically useful threshold, and for the first time provides a mechanism for defining the levels at which leachables (and extractables) should be identified and evaluated. In other words, the AET addresses the question posed repeatedly by OINDP pharmaceutical development scientists:

How low do we go?

The use of analytical thresholds for identifying, reporting, and quantifying drug substance impurities, drug product impurities, and residual solvents is a well established practice in the pharmaceutical industry[1-4]. It is also the experience of the Working Group that arbitrary identification and reporting thresholds are generally employed by individual pharmaceutical development teams for OINDP extractables and leachables, although no existing guidance document suggests such thresholds. In this section, the Working Group proposes an analytical threshold for OINDP leachables and extractables that is scientifically justified, being derived from safety thresholds that are based on safety data and risk assessments.

The following sections describe in detail a proposed process for establishing an AET for any organic leachables profile. The process considers the significant parameters of an individual OINDP, e.g., doses/day, and the particular analytical techniques/methods used to establish leachables/extractables profiles. It further considers the uncertainty inherent in any particular analytical technique/method. Although the SCT is by definition and design expressed and applied only to leachables, a process is described which not only converts the appropriate safety threshold to an AET for leachables, but also translates it to an AET for extractables. This

process allows the AET to be used in Controlled Extraction Studies on OINDP container closure system critical components, and to involve the toxicology pharmaceutical development team member(s) in the safety evaluation of potential leachables at this important and early phase of an OINDP development program. The early identification and evaluation of potentially toxic leachables provides clear benefits to the efficiency of a pharmaceutical development program, as well as improved OINDP safety and quality.

B. Determination of the AET

The AET is defined as the threshold 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. The process of determining an AET begins with the SCT, and an understanding of the parameters of the OINDP under development. The overall process starting from the SCT, is as follows:

1. Convert the SCT (*0.15 µg/day for an individual organic leachable*) to an *Estimated AET* (*µg/canister for an individual organic leachable in an MDI, for example*) by considering the dosing and other parameters of the particular OINDP.
2. Convert the *Estimated AET* for leachables to an *Estimated AET* for extractables (*µg/g elastomer for an individual organic extractable, for example*) by considering the parameters of the particular OINDP container closure system, e.g., weight of elastomer per MDI valve.
3. Locate the *Estimated AET* on a particular leachables or extractables profile, e.g., a GC/MS Total Ion Chromatogram.
4. Evaluate the uncertainty of the particular analytical technique/method, e.g., GC/MS response factors for various potential extractables/leachables.
5. Convert the *Estimated AET* to a *Final AET* by considering this analytical uncertainty.

In general, overfill should not be considered in the calculations for various OINDP, unless scientifically justified. For example, in some cases the overfill is quite large and is required and justified for technological reasons, and is not implemented only to cover small changes such as variability in filling or loss of propellant/solvent over time.

Each step of the process is more fully described below.

Note that the calculations and resultant AET levels presented below are examples, and are given in order to illustrate how AETs for various OINDP might be calculated. They are not meant to be prescriptive.

1. Estimated AET – MDI (Metered Dose Inhaler) Example

Metered Dose Inhalers represent arguably the "worst case scenario" for correlation of leachables with extractables, i.e., all chemical entities that are observed as extractables from critical elastomeric and plastic dose metering valve components are also observed as leachables in drug product. Further, leachables observed in MDI formulations at accelerated storage

conditions and/or near the end of shelf-life are always present at significant concentration levels relative to the corresponding extractables concentration levels in valve components (assuming that Controlled Extraction Studies, Leachables Studies, and Routine Extractables Testing are accomplished correctly). The MDI, therefore, is the OINDP which has the highest probability of exposing a patient to leachables at relatively significant levels, and it is appropriate to identify and evaluate leachables at the lowest level of potential safety concern.

The Working Group recommends that AETs for MDI leachables profiles be based on the Safety Concern Threshold (SCT) of 0.15 µg/day for an individual organic leachable. This recommendation includes potential organic leachables derived from critical components of the dose metering valve, canister inner surface, and inner surface coating if present.

The SCT represents an absolute daily intake value, which will not vary based on the daily dosing regimen or other parameters of a particular OINDP. For practical use in the laboratory, i.e., for Controlled Extraction Studies and Leachables Studies, a threshold must be defined in relative terms, such as mass of an individual extractable per mass of critical component, for extractables studies, or mass of an individual leachable per product or dose, e.g., µg/canister, for leachables studies. Thus, the *Estimated AET* is determined by simply converting the SCT from units of daily exposure to these OINDP relative units.

For example, consider an MDI with 200 *labeled* actuations per canister, a recommended dose of 12 actuations per day, and a critical component elastomer mass per valve of 200 mg. For an individual organic leachable derived from this elastomer, the estimated AET would be:

$$\textit{Estimated AET} = \left(\frac{0.15\ \text{mg/day}}{12\ \text{actuations/day}} \times 200\ \textit{labeled}\ \text{actuations/canister} \right)$$

$$\textit{Estimated AET} \approx 2.5\ \text{mg/canister}$$

Converting to an *Estimated AET* for individual extractables in an extractables profile of this particular elastomer:

$$\textit{Estimated AET} \approx \frac{(2.5\ \text{mg/canister}) \times (1\ \text{canister/valve})}{0.2\ \text{g elastomer/valve}}$$

$$\textit{Estimated AET} \approx 12.5\ \text{mg/g}$$

In the experience of the Working Group, this example *Estimated AET* is typical of current pharmaceutical development practice.

Note: The above calculation assumes that all 200 mg of elastomer in this particular MDI valve has the same chemical composition and extractables profile, and takes no account of the number of individual valve components fabricated from this elastomer. When accomplishing

Controlled Extraction Studies and establishing acceptance criteria for unspecified, i.e., "new," extractables in Routine Extractables Testing programs, the pharmaceutical development team should consider the potential additive effect to the leachables profile of multiple elastomeric and/or plastic components fabricated from the same basic material.

Due to factors such as variability in filling and loss of propellant/solvent over the shelf-life of the product, the calculation of the *Estimated AET* should not be modified by considerations of overfill at manufacture, unless scientifically justified (for example, in some cases the overfill is quite large and is required and justified for technological reasons, and is not implemented only to cover small changes such as variability in filling or loss of propellant/solvent over time). In general, the number of actuations guaranteed by label claim at the end of the product's shelf-life should be entered into the calculation. The number of actuations per day should also be the highest for the particular drug product based on proposed labeling information. It is also considered inappropriate to use other adjustment factors to modify the *Estimated AET*, for example an adjustment factor based on valve delivery versus delivery from the mouthpiece.

Note: Such adjustment factors are not only inappropriate for MDI drug products, but for all OINDP. The "worst case scenario" based on labeling information for the OINDP under consideration should be applied.

In addition to leachables derived from critical components of the valve, it is important to consider organic residues, e.g., drawing oils, lubricating oils, cleaning agents, potentially covering metal surfaces, such as the inner surface of the canister or metal valve components. Potential leachables from the canister are of particular concern when the canister has a purpose added organic coating on its inner surface. An *Estimated AET* for organic residues and potential leachables derived from organic coatings should also be calculated based on the considerations outlined above.

Unlike critical valve components or the canister inner surface, the MDI actuator/mouthpiece is unlikely to contribute leachables to the emitted drug product dose. The actuator/mouthpiece is, however, in contact with the patient's mouth during use of the MDI and it is therefore appropriate to accomplish extractables evaluations of this component, including Controlled Extraction Studies and the development of routine tests and acceptance criteria for qualitative and quantitative extractables profiles. An AET for extractables is therefore required for the MDI actuator/mouthpiece for use in Controlled Extraction Studies and routine extractables profile control methods and tests. However, as stated in the FDA draft guidance document for MDI and DPI drug products:[5]

> *"Safety concerns will usually be satisfied if the materials in the components meet food additive regulations and the actuator meets the USP Biological Reactivity Tests (USP <87> and <88>)."*

Based on these considerations:

The Working Group recommends that MDI actuator/mouthpieces have an extractables Estimated AET of 20 µg/g for an individual organic extractable. Adequate extraction conditions should be used (see Chapter II).

This *Estimated AET* is in the same order of magnitude as that for critical MDI valve components in the example presented above, and is sufficient to characterize known chemical additives as well as many relatively minor extractables in an actuator/mouthpiece polymer formulation. This level of extractables characterization will help verify that the FDA's indirect food additive regulations have been met, will help confirm the original stated composition, and will also establish a baseline for identification which will allow for the development and implementation of effective routine control methods for actuator/mouthpiece extractables profiles.

2. Estimated AET – Nasal Spray Drug Product Example

Nasal Sprays and Inhalation Sprays are similar to MDIs in that they are all within the general category of drug/device combinations for oral or nasal inhalation where the device meters the dose. Since the majority of these OINDP include aqueous based formulations, the probability of detecting leachables at significant levels is low relative to MDIs with organic propellant based formulations. However,

The Working Group recommends that AETs for Nasal Spray and Inhalation Spray leachables profiles be based on the Safety Concern Threshold (SCT) of 0.15 µg/day for an individual organic leachable. This recommendation includes potential organic leachables derived from the container and other critical components of the container closure system.

For example, consider a Nasal Spray with 120 *labeled* actuations per container and a recommended dose of 4 actuations per day. For an individual organic leachable the estimated AET would be:

$$Estimated\ AET = \left(\frac{0.15\ mg/\text{day}}{4\ \text{actuations/day}} \times 120\ labeled\ \text{actuations/container} \right)$$

$$Estimated\ AET \approx 4.5\ mg/\text{container}$$

Given a total fill volume of 10 mL (for example), this converts to:

$$Estimated\ AET = \left(\frac{4.5\ mg/\text{container}}{10\ \text{mL/container}} \right)$$

$$Estimated\ AET \approx 0.45\ mg/\text{mL}$$

In the experience of the Working Group, this example *Estimated AET* is well within the capabilities of modern analytical techniques and methods.

As for the MDI, this *Estimated AET* for leachables can be translated into an *Estimated AET* for extractables from critical components of the container closure system that are in continuous contact with the drug product formulation. For example, consider a low density polyethylene tube weighing 250 mg which is a critical component in a Nasal Spray container closure system:

$$Estimated\ AET = \left(\frac{4.5\ mg/container}{0.250\ g\ tube/container} \right)$$

$$Estimated\ AET \approx 18\ mg/g$$

This *Estimated AET* is also in the same order of magnitude as that for critical MDI valve components in the example presented above. For critical components that are not in continuous contact with the drug product formulation:

The Working Group recommends that critical components of Nasal Spray and Inhalation Spray drug product container closure systems that are not in continuous contact with the drug product formulation have an extractables Estimated AET of 20 µg/g for an individual organic extractable.

For nasal sprays and inhalation sprays, critical components include components that are in constant contact with the formulation and components that are in the liquid pathway during actuation of the device, for example, and that do not permit quick evaporation of residual surface liquid (see Chapter I, also see reference 6).

This proposal provides the same level of extractables characterization and control as provided for the MDI actuator/mouthpiece.

3. Estimated AET – DPI (Dry Powder Inhaler) Example

Of all OINDP, the DPI has the lowest probability of exposing a patient to leachables at relatively significant levels. The reasons for this are:

1. The DPI drug product formulation is (obviously) a dry powder and contains no solvent, either organic or aqueous, which can promote leaching of organic chemical entities.

2. The drug product unit dose is most often contained in a separate container closure system, e.g., blister pack or capsule, and is only in contact with critical components of the device itself for a brief period of time.

The most likely source of leachables would be the material composing the unit dose

container, such as a foil laminate blister. Leaching would have to occur via either direct contact of the drug product powder with the container closure material, via volatilization of organic chemical entities from the container closure material with deposition on the dry powder, or via migration of organic chemical entities through the primary packaging material with deposition on the dry powder. The possibility of observing leachables from the DPI unit dose container is best evaluated with detailed Controlled Extraction Studies on the container material to identify potential leachables which could possibly migrate to the dry powder by either solid-solid contact or volatilization, and/or have potential safety concerns.

The Working Group recommends that AETs for Dry Powder Inhaler leachables profiles be based on the Safety Concern Threshold (SCT) of 0.15 µg/day for an individual organic leachable. This recommendation includes organic leachables derived from the unit dose container closure system and other critical components of the device which may have continuous long term contact with the drug product formulation.

Leachables studies (either stability studies or "one-time" characterization studies) would only be required for DPIs if potential leachables, i.e., extractables, of safety concern were identified at the AET level during comprehensive Controlled Extraction Studies (see Chapter II).

Consider a DPI containing 13 mg of drug product formulation in a unit dose blister with 50 mg of blister material either in direct contact with the formulation or capable of volatilizing leachables into the headspace above the formulation, and a recommended dose of 2 actuations per day. For an individual organic leachable the estimated AET would be:

$$Estimated\ AET = \left(\frac{0.15\,mg/day}{2\ doses/day} \times 1\ dose/blister \right)$$

$$Estimated\ AET \approx 0.075\,mg/blister$$

Converting relative to the total mass of drug product in a blister:

$$Estimated\ AET = \left(\frac{0.075\,mg/blister}{0.013\,g\ drug\ product/blister} \right)$$

$$Estimated\ AET \approx 5.8\,mg/g\ drug\ product$$

Converting to an *Estimated AET* for extractables from the blister material:

$$Estimated\ \ AET = \left(\frac{0.075\ mg/\text{blister}}{0.050\ \text{g material/b lister}} \right)$$

$$Estimated\ \ AET \approx 1.5\ mg/\text{g blister material}$$

For critical components that are not in continuous contact with the drug product formulation:

The Working Group recommends that critical components of DPI drug product container closure systems that are not in continuous contact with the drug product formulation have an extractables Estimated AET of 20 µg/g for an individual organic extractable.

Note that comprehensive Controlled Extraction Studies should always be performed on non-contact DPI critical components using the AET, even if they do not have continuous long term contact with the drug product formulation.

This proposal provides for the same level of extractables characterization and control as provided for the MDI actuator/mouthpiece and Nasal Spray/Inhalation Spray non-contact critical components.

4. Estimated AET – Inhalation Solution Example

Inhalation Solutions are similar to Nasal Spray/Inhalation Spray drug products in that they are most often based on aqueous formulations, and therefore the risk of detecting organic leachables at significant levels is relatively low. Leaching can potentially occur from the unit dose container, e.g., low density polyethylene, which is in long term continuous contact with the drug product formulation. It is also possible that organic chemical entities associated with paper labels, adhesives, inks, etc. in direct contact with the unit does container can migrate into the drug product formulation.

The Working Group recommends that AETs for Inhalation Solution leachables profiles be based on the Safety Concern Threshold (SCT) of 0.15 µg/day for an individual organic leachable. This recommendation includes potential organic leachables derived from the unit dose container closure system and other materials which may have continuous long term contact with the drug product formulation or unit dose container.

Consider an Inhalation Solution with 3 mL of drug product contained in a low density polyethylene (LDPE) container (1 g total weight LDPE), with a recommended dose of 3 containers per day. For an individual organic leachable the estimated AET would be:

$$Estimated\ AET = \left(\frac{0.15\ mg/day}{3\ doses/day} \times 1\ dose/container \right)$$

$$Estimated\ AET \approx 0.05\ mg/container$$

$$Estimated\ AET = \left(\frac{0.05\ mg/container}{3\ mL/container} \right)$$

$$Estimated\ AET \approx 0.017\ mg/mL$$

Converting to an *Estimated AET* for individual extractables in an extractables profile of this particular LDPE:

$$Estimated\ AET = \left(\frac{0.05\ mg/container}{1\ g\ material/container} \right)$$

$$Estimated\ AET \approx 0.05\ mg/g\ container\ material$$

The Working Group recognizes that the proposed leachables/extractables *Estimated AET* for Inhalation Solution drug products represents a significant analytical challenge to an OINDP pharmaceutical development team. Therefore,

The Working Group recommends that if it can be scientifically demonstrated that:

1. ***Aqueous and/or drug product formulation extracts of Inhalation Solution direct formulation contact container closure system material yield no extractables at Final AET levels, or no extractables above final AET levels with safety concern; AND***
2. ***There is no evidence for migration of organic chemical entites through the unit dose container into the drug product formulation; THEN***

Drug product leachables studies are not required.

This recommendation implies:

1. Careful and comprehensive Controlled Extraction Studies using water as well as stronger solvents such as methylene chloride or 2-propanol to identify any potential leachables, i.e., extractables, of potential safety concern.
2. A well designed drug product without paper labels and other sources of organic chemical migration into the drug product, either from the environment or from secondary protective packaging.

3. Comprehensive and fully validated Routine Extractables Testing methods, capable of detecting any significant change in the unit dose container material extractables profile.

Additional discussion of this recommendation is presented in Chapter III, which addresses leachables studies.

5. Final AET

Obviously, if one is able to accurately quantitate every individual leachable (or extractable) in a particular profile then the *Estimated AET* is exactly equal to the *Final AET*. For leachables profiles this in fact might be the case since comprehensive Controlled Extraction Studies would have been accomplished, providing identifications of all potential leachables and ample time to develop and validate quantitative leachables methods with all appropriate reference compounds. Given a properly accomplished Controlled Extraction Study and a thorough understanding of manufacturing processes, the detection of a completely unknown leachable during drug product stability studies should be a rare occurrence, although not impossible. During Controlled Extraction Studies, however, where it is not practical to accurately quantitate each and every individual extractable with an authentic reference compound, the *Estimated* and *Final AETs* are important thresholds which serve to rationalize the overall scope of the study.

The *Estimated AET* can be located on a particular extractables/leachables profile, e.g., GC/FID chromatogram, GC/MS Total Ion Chromatogram, LC/UV chromatogram, relative to the response of an appropriately selected internal standard (see discussion below), or the response(s) of authentic reference compounds representing *Confirmed* identifications of major extractables/leachables. The *Final AET* can then be determined by incorporating into the *Estimated AET* a factor that reflects the uncertainty inherent in any particular analytical method. Analytical uncertainty is a result of the differing responses that chemical entities with different molecular structures have with analytical techniques/methods. This analytical uncertainty is of particular significance for leachables and extractables which, as previously discussed, can represent a wide variety of chemical classes and molecular structure types. The *Final AET* is, therefore, dependent on the analytical technique(s)/method(s) used to create the extractables/leachables profile(s) being investigated.

One possible approach to accomplishing an evaluation of analytical uncertainty is through the use of Response Factors (RFs). A Response Factor is defined as:

$$RF = A_a/C_a$$

Where: A_a = Response of an individual analyte, e.g., chromatographic peak area
C_a = Concentration (or mass) of the individual analyte

For a GC/MS method, for example, the chromatographic peak areas for individual analytes, i.e., leachables or extractables, as determined from either the Total Ion Chromatogram (TIC) or individual mass chromatograms (extracted ion current profiles), are divided by individual analyte concentrations in a known sample of authentic reference compounds. The concentration levels of the authentic reference compounds chosen for RF determination must be within the linear

dynamic range of the analytical system. For GC/MS this means not overloading the GC column or saturating the mass spectrometer's detector. A somewhat more precise uncertainty evaluation can be obtained through the use of Relative Response Factors (RRFs), which are defined as follows:

$$RRF = C_{is}A_a/A_{is}C_a$$

Where: C_{is} = Concentration (or mass) of an internal standard
A_{is} = Response of the internal standard
A_a = Response of an individual analyte
C_a = Concentration of the individual analyte

The RRF normalizes individual RFs to the RF of an internal standard. The use of internal standards is a well established procedure for improving the accuracy and precision of trace organic analytical methods.

The Working Group recommends that analytical uncertainty be evaluated in order to establish a Final AET for any technique/method used for detecting and identifying unknown extractables/leachables.

A summary of the process discussed above as one way to evaluate analytical uncertainty is as follows:

1. Given a particular extractables/leachables profile obtained by a particular analytical technique/method, create a list of individual analytes which have *Confirmed* identifications and for which authentic reference compounds are available.

   This analyte list should ideally include chemical entities representing all known ingredients in the appropriate container closure system component(s), and all identified molecular structure classes of extractables/leachables that were not stated explicitly in the ingredients, e.g., *specific alkanes that constitute the general ingredient "paraffins."*

2. Choose an internal standard appropriate to the particular analytical technique/method.

   Some characteristics of a good internal standard are:

   - *It should be compatible with the particular analytical technique.*
   - *It should be "well-behaved" in the particular analytical method. A "well behaved" internal standard in a GC method, for instance, will not have a significant tailing factor, will not irreversibly adsorb onto the column, etc.*
   - *It should be stable in the analytical matrix.*
   - *It should not be interfered with by other analytes or components in the analytical matrix.*

- *It should possess a response similar to those of other analytes in the particular analytical technique/method.*

3. Analyze a mixture(s) of authentic reference compounds with the internal standard using the particular analytical technique/method.

   This analysis should be accomplished according to principles of sound scientific practice, e.g., at appropriate concentration levels, with an appropriate number of replicates, with appropriate blanks and controls.

4. Calculate RRFs for all analytes and create an RRF database.

   An example of a Relative Response Factor database is presented in Table 1. This database was created by the Working Group using a GC/FID (Gas Chromatography/Flame Ionization Detector) analytical method, and the extractables were arbitrarily selected so that the extractables chosen are not representative of the test article extractables profiles acquired by the Working Group.

5. Calculate statistical parameters for the RRF database, including the Standard Deviation (SD) and %Relative Standard Deviation of RRFs.

   The analytical uncertainty can then be estimated based on this database and statistical parameters.

The Working Group proposes and recommends that analytical uncertainty in the Estimated AET be defined as one (1) %Relative Standard Deviation in an appropriately constituted and acquired Response Factor database OR a factor of 50% of the Estimated AET, whichever is greater.

The *Estimated AET* is then reduced by the uncertainty factor to yield the *Final AET* for the particular extractables/leachables profile.

For example, consider the *Estimated AET* for the hypothetical Metered Dose Inhaler presented above:

$$\textit{Estimated AET} \approx 2.5\textit{mg}/\text{canister}$$

Given the Response Factor database in Table 1, the *Final AET* would be:

$$\textit{Final AET} = 2.5\textit{mg}/\text{canister} - 0.29(2.5\textit{mg}/\text{canister})$$

$$\textit{Final AET} = 1.8\textit{mg}/\text{canister}$$

Note that 50% of a 2.5 µg/canister is 1.3 µg/canister which is lower than 1.8, and therefore:

4413
4414
4415 $Final\ AET = 1.3 mg/\text{canister}$
4416

Table 1. Example Extractables RRF Database from GC/FID Method. 2-Fluorobiphenyl as internal standard

Analyte ID	RF Value	RRF Value
BHT	19.28	0.95
Irganox 1076	7.4	0.35
p-terphenyl-D14	17.40	0.88
Bis (2-ethylhexyl) phthalate	14.38	0.71
2,6-d-tert-butylphenol	19.96	0.96
Eicosane	15.73	0.77
Diphenylamine	21.91	1.05
Dibutyl phthalate	12.54	0.61
Statistics		
Mean	**16.08**	**0.79**
Standard Deviation	**4.66**	**0.23**
%RSD	**28.98**	**29.00**

4417

4418 **6. Summary of Process to Determine Estimated and Final AET**
4419

4420 The processes for determining both the *Estimated and Final AET* described above are
4421 summarized in a step-wise manner in Table 2. This is only one possible approach to determining
4422 the Final AET. Other scientifically justifiable approaches can be used. The process outlined
4423 below is designed to be general so that it can be applied to various analytical techniques/methods
4424 used for both extractables and leachables profiling.

4425

Table 2. A Possible Process for Determination of Estimated and Final AET

STEP 1	Determine estimated AET by converting SCT (0.15 µg/day) to units relative to an individual OINDP (e.g, µg/canister, µg/gram component, etc.).
STEP 2	Estimate position on the particular extractables/leachables profile of the SCT. *This is the Estimated AET.* The position should be based on: • The RF of an appropriate internal standard; or • The RF of an unambiguously identified major extractable/leachable.
STEP 3	Evaluate analytical uncertainty:

	• Create an appropriate RRF database. • Determine the Standard Deviation (SD) and %Relative Standard Deviation (%RSD) of RRFs in the database; • Define the analytical uncertainty: The Uncertainty Factor is equal to (%RSD/100)(Estimated AET) or 0.50(Estimated AET), whichever is greater
STEP 4	Establish the *Final AET* : • The *Final AET* is defined as: Final AET = Estimated AET – "uncertainty factor"

C. Conclusions

The Working Group recognizes that both the AET concept and the process for AET determination have limitations. For example, while it might be relatively easy to determine both *Estimated* and *Final AETs* for extractables/leachables profiles acquired by GC/MS (Gas Chromatography/Mass Spectrometry), GC/FID (Gas Chromatography/Flame Ionization Detection), and LC/UV (Liquid Chromatography/Ultraviolet detection), it might not be so simple for a technique like LC/MS (Liquid Chromatography/Mass Spectrometry) which does not create a readily useable extractables/leachables profile (see previous discussion on LC/MS in Chapter II).

However, in spite of its limitations the AET concept represents a significant reduction in the uncertainty associated with the OINDP pharmaceutical development process. Such uncertainty reductions are a stated goal of the Working Group.

Note: As previously mentioned, the AET concept does not apply to the compounds and compound classes of special safety concern. These include N-nitrosamines, Polynuclear Aromatic Hydrocarbons (PAHs or PNAs), and 2-mercaptobenzothiazole.

References

1 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Impurities in New Drug Substances, Q3A(R1). Available electronically at: http://www.ich.org/LOB/media/MEDIA422.pdf

2 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Impurities in New Drug Products, Q3B(R2). Available electronically at: http://www.ich.org/LOB/media/MEDIA421.pdf

3 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Impurities in Residual Solvents, Q3C(R3). Available electronically at: http://www.ich.org/LOB/media/MEDIA423.pdf

4 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Impurities in Residual Solvents, Q3C(R3). Tables of solvents and Appendix 1, pp. 5-12.

5 Draft Guidance for Industry, Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products – Chemistry, Manufacturing, and Controls Documentation; Draft Guidance for Industry; U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER); Rockville, MD, October 1998; 1-62.

6 Guidance for Industry, Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products – Chemistry, Manufacturing and Controls Documentation; Guidance for Industry; U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER); Rockville, MD, July 2002; 1-45.

PART 4:

APPENDICES

APPENDIX 1: SAFETY CONCERN THRESHOLD CONVERSION TABLES

Table 1. Leachable Concentrations Corresponding to Safety Concern Threshold of 0.15 µg/day

MDI Drug Product	Estimated Formulation Parameters from Product Labeling			Leachable Concentration Yielding 0.15 µg/day Intake	
	Formulation Net Weight (grams)	Number of Actuations Per Can	Maximum Actuations Per Day	(µg/g)	(µg/can)
Flovent 110	7.9	60	8	0.14	1.1
Alupent	7.0	100	12	0.18	1.3
Beconase *	6.7	80	8	0.22	1.5
QVAR	7.3	100	8	0.26	1.9
Nasacort *	9.3	100	8	0.20	1.9
Tilade	16.2	104	8	0.12	2.0
Azmacort	20.0	240	16	0.11	2.3
Proventil HFA	6.7	200	12	0.37	2.5
Ventolin HFA	18.0	200	12	0.14	2.5
Combivent	14.7	200	12	0.17	2.5
Atrovent	14.0	200	12	0.18	2.5
Serevent †	13.0	120	4	0.35	4.5
Maxair	14.0	400	12	0.36	5.0
median	13.0	120	12	0.18	2.3

Leachable concentrations corresponding to 0.15 µg/day intake are estimates calculated from formulation parameters as stated in the US product labeling. **These estimates are for illustrative purposes only and should not be used for decision making because they may not reflect actual MDI formulation parameters**.

Leachable µg/can at 0.15 µg/day = 0.15 µg/day × Actuations/can ÷ Actuations/day

Leachable µg/g at 0.15 µg/day = µg/can ÷ Net Formulation Weight

* Nasal inhalation drug product.

† No longer marketed in US.

Table 2. Leachable Concentrations Corresponding to Qualification Threshold of 5 µg/day

MDI Drug Product	Estimated Formulation Parameters from Product Labeling			Leachable Concentration Yielding 5 µg/day Intake	
	Formulation Net Weight (grams)	**Number of Actuations Per Can**	**Maximum Actuations Per Day**	(µg/g)	(µg/can)
Flovent 110	7.9	60	8	4.7	38
Alupent	7.0	100	12	6.0	42
Beconase *	6.7	80	8	7.5	50
QVAR	7.3	100	8	8.6	63
Nasacort *	9.3	100	8	6.7	63
Tilade	16.2	104	8	4.0	65
Azmacort	20.0	240	16	3.8	75
Proventil HFA	6.7	200	12	12.4	83
Ventolin HFA	18.0	200	12	4.6	83
Combivent	14.7	200	12	5.7	83
Atrovent	14.0	200	12	6.0	83
Serevent †	13.0	120	4	11.5	150
Maxair	14.0	400	12	11.9	167
median	13.0	120	12	6.0	75

Leachable concentrations corresponding to 5 µg/day intake are estimates calculated from formulation parameters as stated in the US product labeling. **These estimates are for illustrative purposes only and should not be used for decision making because they may not reflect actual MDI formulation parameters.**

Leachable µg/can at 5 µg/day = 5 µg/day × Actuations/can ÷ Actuations/day

Leachable µg/g at 5 µg/day = 5 µg/can ÷ Net Formulation Weight

* Nasal inhalation drug product.

† No longer marketed in US.

APPENDIX 2

EXAMPLES OF LEACHABLES

Some representative compounds that may be found as leachables in an MDI are shown in Table 1. These compounds would be derived from the elastomeric and polymeric components of the MDI valve. Potential levels of these compounds that could be found in a representative MDI are also shown, based on the experience and knowledge of Working Group members. *This list is not designed to be comprehensive, but only representative.*

Note that the range and levels of leachables would be significantly decreased for products such as DPIs and nasal sprays.

Table 1. Examples of Leachables Found In OINDP, and Their Typical Levels in a Representative MDI[a]

Extractable	Levels (amount per canister)	Levels (TDI)
Sulfur-containing compounds Tetramethylthiourea 2-mercaptobenzothiazole Tetramethylthiuramdisulfide Zinc tetramethyldithiocarbamate	**1-100 mg/canister**	**0.05-5 mg TDI**
Phenolic antioxidants Butylatedhydroxytoluene Irganox 1010 Irganox 1076 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl] phenol	**50-500 mg/canister**	**2.5-25 mg TDI**
Amine antioxidants Diphenylamine	**50-500 mg/canister**	**2.5-25 mg TDI**
Phthalate plasticizers Dibutylphthalate Di-n-octylphthalate Di-2-ethylhexylphthalate Didodecylphthalate	**50-500 mg/canister;**	**2.5-25 mg TDI**

Glycol ester plasticizers Triethyleneglycoldicaprate Triethyleneglycoldicaprylate Triethyleneglycolcaprate-caprylate	**50-500 mg/canister;**	**2.5-25 mg TDI**
Fatty acid plasticizers Stearic acid Palmitic acid Myristic acid	**50-500 mg/canister**	**2.5-25 mg TDI**
Nitrosamines[b] N-nitrosodimethylamine N-nitrosodiethylamine N-nitrosodi-n-butylamine N-nitrosopiperidine N-nitrosopyrrolidine N-nitrosomorpholine	**1-100 ng/canister**	**0.05-5 ng TDI**
PNAs[b] Naphthalene Acenaphthylene Fluorene Phenanthrene Anthracene Pyrene Benzo(a)anthracene Chrysene Benzo(b)fluoranthene Benzo(k)fluoranthene Indeno(1,2,3-cd)pyrene Benzo(a)pyrene Benzo(e)pyrene Dibenzo(ah)anthracene Benzo(ghi)perylene	**1-50 mg/canister;**	**0.05-2.5 mg TDI**

a. As an ***example*** for MDIs, the following assumptions were considered in order to calculate the TDI values shown in Table 4: 200 actuations/canister; 2 actuations/dose; 5 doses/day; 10 actuations/day; 50 μL/actuation (total drug delivered through the valve)

b. Note that PNAs and nitrosamines are considered special cases that should be controlled by thresholds other than the ones being developed here.

Table 2 contains a list of representative extractables that can be found in polymers that may constitute components or primary packaging for OINDP. The polymers are related to primary containers, laminates, adhesives, coatings and processing materials. Extractable information is given since these chemical entities represent several different types of packaging in contact with different types of drug formulations. *This list is only a sampling and is not comprehensive for all packaging systems.*

Some of these species have been detected as leachables in drug products, and in predictive modeling studies or have shown up unexpectedly in drug product chromatograms. The estimated amounts range from 0.01 μg – 1000 μg per packaging component or more. The TDI can be calculated from these amounts. Leachable type and concentration will depend on the drug product, drug product formulation, and packaging, e.g., MDI, DPI, nasal spray, solvent, and size of package.

Table 2. Representative Extractables from Polymers

Extractable	**CAS Number**
Solvents	
methanol	67-56-1
ethanol	64-17-5
butanol	71-36-3
ethyl acetate	141-78-6
propylene glycol	57-55-6
methyl ethyl ketone	78-93-3
methyl isobutyl ketone	108-10-1
Monomers/Dimers/Trimers	
methylmethacrylate	80-62-6
butyl/isobutyl acrylates	141-32-2/106-63-8
styrene	100-42-5
formaldehyde	50-00-0
tripropylene glycol di/triacrylate	042978-66-5; 015625-89-5
4,4(1-methylethylidene) bisphenol	86-05-7
Curatives/Photo-initiators	
benzophenone	119-61-9
1-hydroxycyclohexyl phenyl ketone	947-19-3
methyl-o-benzoyl benzoate	116-82-5
Plasticizers	
dipropylene glycol dibenzoate	27138-31-4
dicyclohexyl phthalate	84-61-7

Lubricants/Processing Aids	
oleamide	301-02-0
erucamide	12-89-5
ethylene bistearamide	110-31-6
bis (2-ethylhexyl) adipate	103-23-1
epoxidized soybean oil	8013-3-07-8
silicone oil	069430-45-1
triethanol amine	102-71-6
pentaerithritol	115-77-5
dehydroabietic acid	008050-09-7
Antioxidants	
triethylene glycol bis(3-(3-tertbutyl-4-hydroxy-5 methyl phenyl propionate))	36443-68-3
tris (2,4-di-tert-butyl phenyl) phosphite	315-70-04-4
tris(nonylphenyl) phosphite	26523-78-4
2-hydroxy-4-(octyloxy) benzophenone	1843-05-6
didodecyl 3,3'-thiodipropionate	123-28-4

4528

APPENDIX 3

EXAMPLE OF LEACHABLES RISK ASSESSMENT AND SAR ANALYSIS

I. INTRODUCTION

A crucial part of the extractables and leachables evaluation for components and drug product includes exchange of information and expertise among materials experts, chemists and toxicologists regarding the extractables and leachables present in the component or drug product. Input from toxicologists should be obtained during consideration of the types of components/materials used in the OINDP. During extraction studies on components in development and leachables studies on drug product, chemists and toxicologists should consult with one another regarding extractables that are above defined safety thresholds. Therefore, an integrated approach, incorporating materials, analytical and safety expertise should be utilized throughout the pharmaceutical development process. This approach encourages maximum control of extractables and potential leachables and therefore significantly increases the probability that compounds of concern are identified early in the development process, and decreases the likelihood of quality and safety concerns later in the process.

The chemist should communicate analytical information on the leachables or extractables to the toxicologist to permit a preliminary toxicological evaluation. This evaluation should include 3 key steps:

- Identification information on the leachable/extractable should be conveyed by the chemist to the toxicologist;

- The toxicologist should conduct structure-activity relationship (SAR) studies on the identified leachables/extractables as a preliminary check for potential safety risks; and

- The toxicologist should request from the chemist any further identification or dosage information needed in order to perform a rigorous and meaningful risk assessment and qualification of the given leachables/extractables.

The PQRI Leachables and Extractables Working Group performed each of these steps as part of its overall effort to develop a clear process and general recommendations for conducting leachables studies for OINDP, using proposed safety and analytical thresholds and best practices. Because the Working Group did not perform a true leachables study (i.e., stability study on actual drug product), the Working Group conducted an example risk assessment using results from its Controlled Extraction Studies. In these studies, the chemists identified extractables, and provided the identification information to the Working Group toxicologists for structure-activity relationship (SAR) evaluation.

The Working Group toxicologists conducted SAR studies on the extracted compounds using the identification information. These studies were performed in order to provide an example of how chemical data and SAR assessments are used in risk assessment.

We present a general description of the identification information provided by the chemists, a summary of the SAR study results, and an example of how such study results might be used by both chemists and toxicologists in a typical evaluation of leachables/extractables safety risk and leachables qualification. We also provide the decision tree for conducting safety qualification of leachables. This decision tree is included in the *Justification of Thresholds for Leachables in Orally Inhaled and Nasal Drug Products* (see Part 2).

It is important to note that computational toxicology assessments represent a preliminary risk assessment and are only ***a part*** of the overall risk assessment. Such assessments inform the direction of further leachables risk assessment and qualification studies. A process for conducting these further assessments is outlined in the decision tree for conducting safety qualification of leachables. This tree provides guidance on how a sponsor could qualify a given leachable.

II. EXPERIMENTAL BACKGROUND

A. Identification Results

The PQRI Leachables and Extractables Working Group Chemists conducted an optimized extraction study on a sulfur-cured elastomer extracted with methylene chloride under Soxhlet extraction.[1] Sixty-six extractables were identified from GC/MS data. The identification process consisted of obtaining structural information on the compounds, which resulted in assignment of a range of identification categories to the various leachables. For example, some were assigned "confirmed" identification, where data were matched with reference standards; and some were assigned "confident" or "tentative" identification, where identification is increasingly less certain.[2] For instance, "confident" identification would address those instances where one could preclude all but the most closely related compounds, and "tentative" identification would cover identification of the class of molecule.

Note that the degree of identification varies depending on the compound, amount of the compound and the analytical method used. See Part 3, Chapter II, Controlled Extraction Studies, Table 3 for the full list and identification levels of the extracted compounds.

The compounds assigned "confirmed" or "confident" structures were evaluated for structure-activity relationships (SAR) using representative computational toxicology estimations. Two SAR studies were conducted on sets of the confirmed and confident structures. One study was performed by FDA using MultiCase computational toxicology software.[3] The second study was performed by Pfizer Inc., using DEREK computational toxicology software.

Since SAR databases can be used as a first step in providing preliminary information about the safety of a compound, the Working Group chose to assess the structures for carcinogenicity, mutagenicity, and teratogenicity. Note that below the QT, compounds should be assessed for carcinogenic, mutagenic and hypersensitivity potential. Teratogencity becomes more important at levels above the QT.

Results from computational analyses such as DEREK and MultiCase should be considered starting points in the SAR analysis process. Any results from the software should not necessarily be taken at "face value" and will need to be considered in the context of previous experience/data and literature results, to better understand the relevance of the result.

B. Summary of SAR Study Results

Table A2.1 contains summarized results from the SAR studies.

Table A2.1

	Leachable Compound	**MultiCase Alert?**	**DEREK Alert?**
1	α-Methyl Styrene	No	No
2	Indene	No	No
3	Naphthalene	Yes Possible carcinogen	No
4	Tetramethylthiourea	Yes Possible carcinogen, teratogen	Yes Possible carcinogen
5	Benzothiozole	No	Yes Possible carcinogen
6	Ethyl-4-tert-butyl phenyl ether	No	No
7	2,5-di-tert-butylphenol	No	Yes Possible skin sensitization
8	2-methyl-thiobenzothiazole	N/A	Yes Possible carcinogen,
9	2-chloro-methyl-thiobenzothiazole (later determined to be an extraction artifact)	Yes Possible carcinogen, mutagen	Yes Possible carcinogen, mutagen, skin sensitization
10	2-mercaptobenzothiazole	Yes Possible carcinogen	Yes Possible carcinogen, skin sensitization

11	Hexadecanoic acid	Yes Possible teratogen, skin sensitization	Yes Possible carcinogen
12	3,5-bis-1,1-dimethylethyl-4-hydoroxy benzoic acid	N/A	No
13	n-Eicosane	No	No
14	Bis-(4-methylphenyl)disulfide	No call (poor coverage – unknown fragments)	Yes Possible skin sensitization
15	Heneicosane	No	No
16	Linoleic acid	Yes, Possible teratogen, skin sensitization	Yes Possible carcinogen
17	(E)-Octadecenoic acid	Yes, Possible teratogen, skin sensitization	Yes Possible carcinogen
18	Stearic acid	Yes, Possible teratogen, skin sensitization	Yes Possible carcinogen
19	1-Octadecene	No	No
20	n-Docosane	No	No
21	Tricosane	No	No
22	Tetracosane	No	No
23	2,2’-Methylene-bis-(6-tert-butyl)-2-ethylphenol 2,2’-Methylene-bis-(6-tert-butyl)-4-ethylphenol	No	Yes, Possible skin sensitization
24	Pentacosane	No	No
25	Hexacosane	No	No
26	Heptacosane	No	No

27	Octacosane	No	No
28	Nonacosane	No	No
29	Triacontane	No	No

C. Assessment of Results

In general, both studies revealed similar results for each of the compounds, with some differences in interpretation. One main difference appears to be in results for naphthalene (compound 3), where the MultiCase study gave structural alert for carcinogenicity, and the DEREK study gave no structural alerts.

In addition, for several compounds the DEREK and Multicase studies both generated structural alerts, but the types of alerts differed. For example, lineolic, (E)- Octadecenoic, and stearic acid (compounds 16, 17, and 18) had alerts for teratogenicity and skin sensitization in the Multicase study but alerts for carcinogenicity in the DEREK study.

The MultiCase study also included an evaluation of teratogenicity, while the DEREK study did not. 2-methyl-thiobenzothiazole and 3,5-bis-1,1-dimethylethyl-4-hydoroxy benzoic acid (compounds 8 and 12) were assessed via DEREK only.

III. INFORMATION EXCHANGE BETWEEN TOXICOLOGIST AND CHEMIST

SAR evaluations provide a first step in risk assessment of leachables. As in this example, after the chemist provides preliminary identification information to the toxicologist, the toxicologist should conduct an SAR assessment. From this assessment, the toxicologist would determine which compounds have structural alerts. The toxicologist should then conduct a literature search for toxicological information on each compound above the SCT, i.e., all those included in the SAR assessment. Based on the structural alert information from the SAR assessment and the information from the literature search, the toxicologist would decide which of those compounds require further risk assessment. To make this decision, the toxicologist requires two pieces of information from the chemist:

1. Is the level of structural identification sufficient?

2. At what concentration of this leachable would a patient be exposed?

For example, the toxicologist might focus on compound 4, tetramethylthiourea, which shows several possible alerts. The toxicologist should also have performed a literature search on this compound. If little or no literature on this compound is available and/or the available literature supports the structural alert(s), as a first step the toxicologist should ensure that the structural information on the compound is as complete as possible. Ideally, the chemical structure should be identified to the level of "confirmed." However, for some compounds this is not possible. Therefore, identification should be performed to the extent possible. If further identification

provides new and different structural information, the toxicologist should perform another SAR study and literature search on this compound.

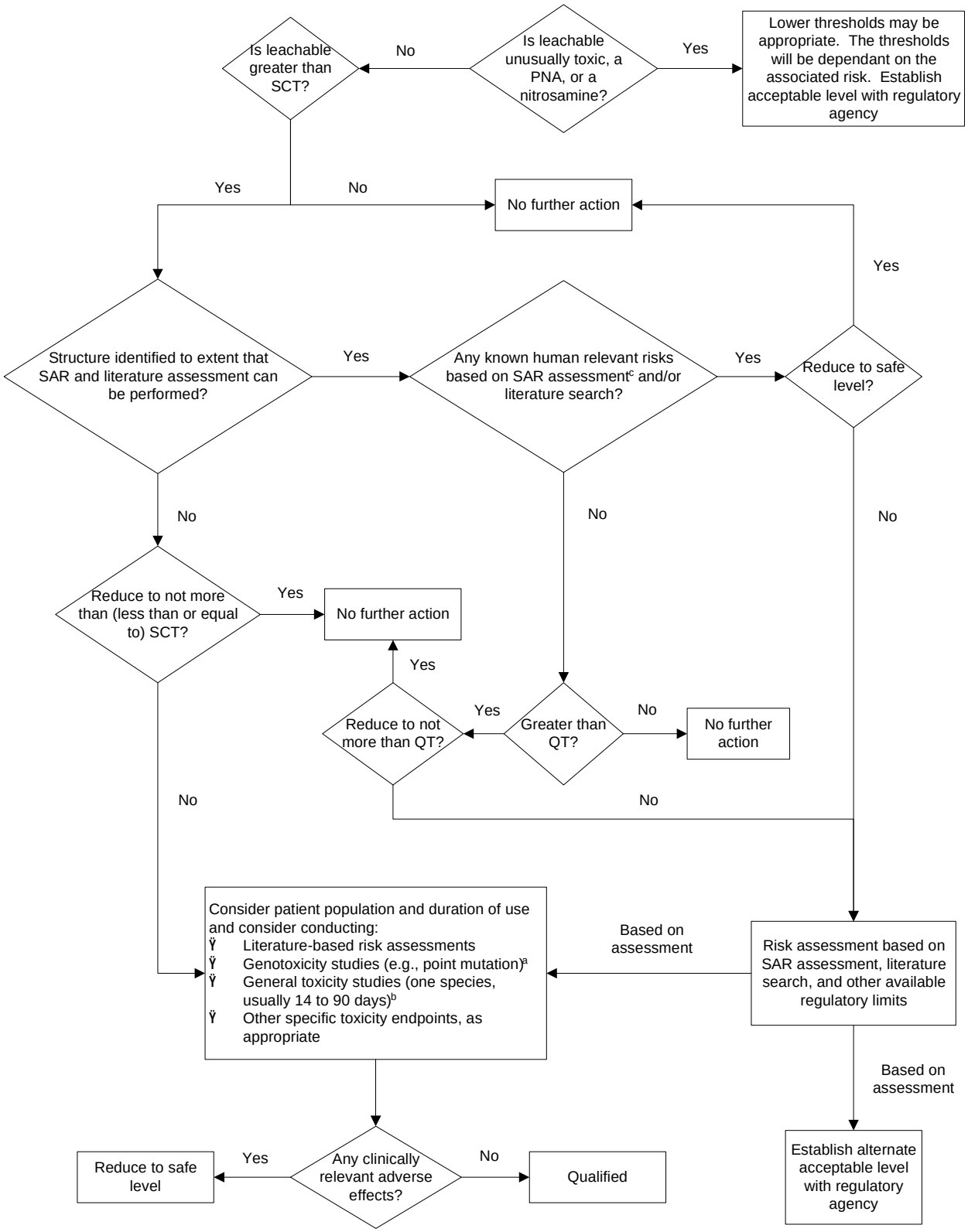
As a next step, the toxicologist should understand the compound concentrations to which a patient would be exposed. She/he would therefore request information on the concentration of the leachable in drug product, the drug product dosage, and the drug product potency from the chemist.

Based on this information, further risk assessment and potential qualification may be performed using the decision tree proposed in the *Justification of Thresholds for Leachables in Orally Inhaled and Nasal Drug Products*, (Figure 1). This decision tree applies the proposed Safety Concern Threshold (SCT) and the Qualification Threshold (QT) for leachables in a safety qualification process. The decision tree is reproduced below for easy reference. If a compound with carcinogenic or genotoxic concerns cannot be reduced to below the SCT, or if very little toxicological literature is available for the compound, the pharmaceutical sponsor chemist and toxicologist should conduct a risk assessment based upon the available information to support the proposed drug product specifications. This risk assessment should then be submitted for review by the FDA counterparts. Based on this review, FDA may accept the proposal, request additional qualification or establish an alternative acceptable level for the compound.

As an example, if the chemist informed the toxicologist that compound 4, tetramethylthiourea (which presents an SAR alert for carcinogenicity) was present in the drug product at a level that would result in a daily human exposure between the SCT and the QT and the literature search confirmed the compound's potential carcinogenicity, the compound should be either reduced to a safe level (below the SCT) or considered for qualification. If the compound cannot be reduced to below the SCT, the pharmaceutical sponsor chemist and toxicologist should perform a risk assessment on the compound based on the available information and the maximum expected level of human exposure through use of the drug product as described above. If the compound were present at levels above the qualification threshold, it would require risk assessment and/or qualification for general toxicologic effects as well as carcinogenic/genotoxic effects. The risk assessment and supporting information should be submitted to FDA for concurrence or a request for further qualification. At levels below the SCT, no action would generally be needed.

As a different example, if the chemist informed the toxicologist that ethyl-4-tert-butyl phenyl ether (which does not present an SAR alert for carcinogenicity, mutagenicity, or sensitization potential) was present in the drug product at a level that would result in a daily human exposure above the QT, the compound should be either reduced to a safe level (below the QT) or considered for qualification. If the compound cannot be reduced to below the QT, the pharmaceutical sponsor chemist and toxicologist should perform a risk assessment on the compound based on the available data and the maximum expected level of human exposure through use of the drug product and/or qualify the compound based on general toxicologic effects. The risk assessment and supporting information should be submitted to FDA for concurrence or a request for further qualification.

4679 **Figure 1. Decision Tree for Safety Qualification**



4680

Footnotes to Safety Qualification Decision Tree

(a) If considered desirable, a minimum screen, e.g., genotoxic potential, should be conducted. A study to detect point mutations, in vitro, is considered an appropriate minimum screen.

(b) If general toxicity studies are desirable, one or more studies should be designed to allow comparison of unqualified to qualified material. The study duration should be based on available relevant information and performed in the species most likely to maximize the potential to detect the toxicity of a leachable. On a case-by-case basis, single-dose studies can be appropriate, especially for single-dose drugs. In general, a minimum duration of 14 days and a maximum duration of 90 days would be considered appropriate.

(c) For example, do known safety data for this leachable or its structural class preclude human exposure at the concentration present?

REFERENCES

1 See Appendix 4 and Part 3, Chapter II Controlled Extraction Studies.

2 See Table 2 in Part 3, Chapter II Controlled Extraction Studies.

3 The MC4PC-ES version of MultiCase was used.

APPENDIX 4

PROTOCOLS FOR CONTROLLED EXTRACTION STUDIES

Leachables and Extractables Working Group

9 January 2003

Experimental Protocol for Controlled Extraction Studies on Elastomeric Test Articles

Submitted to DPTC on 19 November 2002
Approved by DPTC January 2003

TABLE OF CONTENTS

I. INTRODUCTION

In November 1998 and May 1999, the FDA issued two CMC draft Guidances addressing Orally Inhaled and Nasal Drug Products (OINDP): (i) the draft *Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products Chemistry, Manufacturing, and Controls Documentation*[1] (referred to here as the "MDI/DPI draft Guidance"); and (ii) the draft *Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products Chemistry, Manufacturing, and Controls Documentation* (referred to here as the "Nasal Spray draft Guidance"). In July 2002, the Nasal Spray Guidance was finalized.[2]

Currently, both Guidances recommend that the Sponsor identify, report, and conduct toxicological analyses on all extractables found in the controlled extraction study (referred to in the Guidances as a "control extraction study"). Examples of these recommendations are described in the draft MDI/DPI Guidance regarding MDI canisters, valves, and actuators (lines 883-884; 990-991; and 1073):

> *…the profile of each extract should be evaluated both analytically and toxicologically.*

The Product Quality Research Institute (PQRI) Leachables and Extractables Working Group has developed this experimental protocol as an example of a Controlled Extraction Study for elastomeric (i.e., rubber) test articles. Various experimental parameters will be investigated, test article extracts analyzed and results evaluated within the context of the Working Group's approved Work Plan and experimental hypothesis.

This experimental protocol will be used by all laboratories and investigators participating in the study.

II. PURPOSE AND SCOPE OF WORK

A. Purpose

The purpose of the experiments outlined in this protocol is to generate data from Controlled Extraction Studies that will contribute to a larger database, which the Working Group will use to investigate its hypotheses:[3]

1. Scientifically justifiable thresholds based on the best available data and industry practices can be developed for:

   (a) the reporting and safety qualification of leachables in orally inhaled and nasal drug products, and

   (b) reporting of extractables from the critical components used in corresponding container/closure systems.

   Reporting thresholds for leachables and extractables will include associated identification and quantitation thresholds.

2. Safety qualification of extractables would be scientifically justified on a case-by-case basis.

B. Scope

1. Topics Addressed by This Protocol

This protocol covers only Controlled Extraction Studies that would be applied to components from Metered Dose Inhalers (MDIs). The MDI represents the best example of "correlation" between extractables from components and leachables in drug product. Controlled Extraction Studies will be performed following the general outline described in the Guidances. Test articles will be subjected to different extraction conditions to show how different experimentally controlled parameters affect resulting extractables profiles. Additionally, the Working Group will assess experimental results to identify reasonable approaches for sample preparation and analysis of extractables from container and closure components.

As no single analytical technique can be used to identify and quantify all unknown extractables, a variety of methods will be utilized in this protocol to maximize the likelihood that all extractable compounds associated with the test articles are evaluated analytically. Overlap between methods will supply corroborating data that the procedures are valid. To provide a full analytical survey of possible analytes the following strategy will be employed:

1. Direct injection Gas Chromatography/Mass Spectrometry (GC/MS) for identification and assessment of relatively volatile extractables.
2. High Performance Liquid Chromatography/Diode Array Detection (HPLC/DAD) for identification and assessment of relatively polar/non-volatile UV active extractables.
3. High Performance Liquid Chromatography/Mass Spectrometry (LC/MS) for identification and assessment of relatively polar/non-volatile extractables, which may or may not have UV activity.
4. Inductively Coupled Plasma/Mass Spectrometry (ICP/MS), Inductively Coupled Plasma/Atomic Emission Spectroscopy (ICP/AES), and/or Energy Dispersive X-ray (EDX) /Wavelength Dispersive X-ray (WDX) to detect single elements in the extracts (i.e., metals).
5. Fourier Transform Infrared Spectroscopy (FTIR) for characterization of major components in the non-volatile extractable residues.

2. Topics Not Addressed by This Protocol

Studies designed to assess recovery (i.e., mass balance) for individual extractables relative to the known formulations of chemical additives in the elastomeric test articles, or reproducibility of extractables profiles for multiple "batches" of any particular test article are not within the scope of this test protocol.

The extraction procedures, analytical techniques/methods, and analysis conditions described in this experimental test protocol will not be validated as material control methods, since they will be performed in order to collect qualitative information. However, during the course of these experiments the PQRI Leachables and Extractables Working Group will review the results and may initiate additional experimental work for quantitative assessment of extractables.

This protocol does not address system suitability tests for quantitative methods. Appropriate system suitability tests will be addressed later and agreement on this issue will be reached with all of the participating laboratories.

Special case studies such as OVIs (Organic Volatile Impurities), N-nitrosamines or Polynuclear Aromatic Hydrocarbons (PAHs or PNAs) will not be considered in this study. These "special case" classes of extractables have defined and highly specific analytical methods, which are generally accepted and commonly used for their identification and quantitative assessment.

It should be noted that the outlined experimental procedures, analytical instrumentation parameters and conditions, and other details are intended as a guideline for laboratory studies. Details of actual experimental procedures, etc., should be reviewed by the entire group of participating laboratories and investigators so that harmonization between laboratories working on the same test articles can be achieved.

III. REGULATORY STATUS

This is a Good Manufacturing Practices (GMP)[4] study. All experiments shall be performed under GMP conditions to the extent practical in a particular laboratory.[5] Any changes to these protocols shall be documented, following appropriate GMP change control procedures.

IV. SAFETY AND ENVIRONMENTAL IMPACT

Organic solvents are commonly used to enhance solubility of lipophilic targets and to increase transport of small molecules out of complex matrices. These solvents may be flammable and/or show short-term and long-term environmental health risks. Care must be exercised with their use. Consult the Material Safety and Data Sheet (MSDS) for appropriate personal protection and disposal.

V. TEST ARTICLES

Elastomeric materials will be provided in sheet form for use as test articles. The additive formulations and manufacturing conditions for these test articles are known and will be provided to all laboratories participating in the study at the appropriate times.

Note that reference compounds and additive mixtures may be required for the completion of this test protocol and will be provided as appropriate.

VI. CHEMICALS AND EQUIPMENT

Extraction and analytical methods have been chosen and designed so as to utilize chemicals, apparatus, and instrumentation available in typical laboratories routinely involved with this type of study.

A. Extraction Solvents

Extractions will be performed on each test article using three solvents representing a range of polarity selected from the list below. The solvents should be American Chemical Society (ACS) grade or better:

- methylene chloride (dichloromethane)
- 2-propanol (isopropanol)
- hexane (n-hexane, not hexanes)

Depending on the behavior of the test articles in these particular solvent systems, additional solvents may be chosen. Changes in extracting solvent will be discussed by all study participants prior to change initiation by any particular study participant or laboratory.

B. Extraction Apparatus

- Soxhlet apparatus with an Allhin condenser, flask (500 mL), and hot plate or heating mantle
- Sonicator
- Reflux apparatus consisting of an Erlenmeyer flask (125 mL or larger) and condenser with ground glass joints, hot plate or heating mantle.

C. Analytical Instrumentation

- Gas chromatograph equipped with a Flame Ionization Detector (GC/FID)
- Gas chromatograph equipped with a Mass Spectrometer (GC/MS)
- Liquid chromatograph equipped with a photodiode array detector
- Liquid chromatograph equipped with an APCI (Atmospheric Pressure Chemical Ionization) capable Mass Spectrometer (LC/MS)
- Fourier Transform Infrared spectrometer (FTIR)
- EDX and/or WDX equipped with a microprobe or scanning electron microprobe

· Inductively Coupled Plasma Mass Spectrometry (ICP/MS) and/or Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP/AES)

VII. EXTRACTION PROCEDURES

For each extraction technique and solvent type, appropriate blanks (no test article sample) must be prepared. These must be prepared concurrently using a different extraction apparatus (same type) under the same conditions, or by using the same apparatus prior to charging with sample.

Note that the extraction parameters and conditions outlined below are subject to modification and the details of any particular extraction process must be agreed to between all laboratory study participants prior to initiation of experimental work in any particular laboratory.

A. Soxhlet Extraction

1. Sample Preparation

Samples of each test article should be cut into strips appropriately sized to fit into pre-extracted Soxhlet cellulose thimbles. Sample amounts may be in the range of 1-3 g (2 g) using 200 mL of solvent. For quantitative measurements, extracts prepared by Soxhlet will have to be evaporated to dryness and the resulting residues re-dissolved to a known volume (25-50 mL). Alternatively an internal standard can be used for quantitative measurements.

2. Extraction Conditions

Under normal laboratory conditions, three physical extraction parameters may be modified, turnover number, total extraction time and temperature. Temperature is the most difficult of the three parameters to control as the sample holder is maintained above the vapor level (temperature may be above the boiling point), but will be continuously bathed in freshly distilled solvent (coil temperature). It is recommended that the coil temperature be kept as low as possible to avoid heating above the solvent flashpoint.

Turnover number is controlled by the heating rate and should be limited by safety concerns. At low turnover numbers, the extraction characteristics will resemble those of reflux and may be limited by equilibrium phenomena. It is recommended that turnover numbers to be at least ten during the course of the extraction.

Extraction time should be in the range of 24 hours to guard against possible degradation of thermally labile or reactive compounds.

B. Reflux

Reflux extraction is a common and easily implemented approach for the production of extractables (e.g., USP <381> “Elastomeric Closures-Physicochemical Tests”). Conditions are easily standardized as the temperature and pressure are at the defined

boiling points of the extraction solvents. Unlike Soxhlet extraction, reflux extraction is an equilibrium phenomenon.

1. Sample Preparation

Transport of extractables out of the complex matrix may be affected by the surface area and thickness of the test article. Test articles will be prepared by two methods: grinding and cutting into strips appropriately sized to fit into the reflux apparatus.

Sample amounts should be in the range of 2 g using 25–50 mL of solvent. For quantitative measurements the solvent with sample and flask can be weighed and returned to original weight after extraction. Alternatively an internal standard can be used for quantitative measurements.

In reflux extraction, the sample to solvent ratio may affect the completeness of the technique. This should be addressed when optimizing the method for measurement of extractables

2. Extraction Conditions

The only adjustable physical parameter for reflux extraction is time. Extraction time should be 2 to 4 hours. The solvent reservoir level must be monitored and periodically recharged to provide the correct amount of solvent.

C. Sonication

Sonication uses ultrasonic energy instead of thermal energy to increase the rate of diffusion of small analytes out of a solid matrix. Similar considerations as reflux extraction (equilibrium conditions) should be evaluated, but these cannot be calculated using thermodynamic parameters. Sonication equipment may be standardized by measuring the temperature rise after a set exposure time and evaluating the energy deposited into the solvent. Standardization of conditions should be accomplished after consultation between participating laboratories.

1. Sample Preparation

Transport of extractables out of the complex matrix may be affected by surface area and thickness of the test article. Test articles will be prepared by two methods: grinding and cutting into strips appropriately sized to fit into the sonication apparatus.

In sonication, the sample to solvent ratio may affect the completeness of the technique. Therefore, a weight ratio of at least 20:1 solvent to sample should be maintained with sample amounts of 2 g.

2. Extraction Conditions

The only adjustable physical parameter for sonication is time. Bath temperatures should be standardized using either ice-water (0 °C), or monitored by a calibrated thermometer.

Extractions may be completed in as little as 15 minutes. Safety considerations are paramount as extractions are performed under normal atmosphere and the technique may provide easy ignition. The solvent reservoir level must be periodically recharged to provide the correct amount of solvent.

VIII. ANALYTICAL METHODS

A. Chromatographic Methods System Suitability for Extractables Profiling (Qualitative Analyses)

Standard reference materials will be used for qualitative chromatographic analytical techniques to ensure system suitability. The standard reference materials are selected to represent a range of common extractable compounds found in polymeric materials. No one analytical technique is suitable for detection of all targets. The following table presents a list of system suitability analytes for GC and HPLC based analytical techniques. The presence of these analytes should be verified at the recommended concentrations prior to analysis of test article extracts by any participating laboratory.

Note that the entire group of participating laboratories and scientists will judge whether a given participating laboratory has met system suitability for its analytical techniques prior to that laboratory analyzing test article extracts.

Compound Name	Suggested Techniques	Recommended Target Concentration (µg/mL)
Pyrene	GC and LC/UV	1
2-Mercaptobenzothiazole	GC or LC	50
Tetramethylthiuramdisulfide	GC and LC/UV	50
Butylatedhydroxytoluene (BHT)	GC or LC	50
Irganox 1010	LC	50
Diphenylamine	LC	50
Bis (2-ethylhexyl) phthalate	GC or LC	50
Bis (dodecyl) phthalate	GC or LC	50
Stearic acid	GC and LC/MS	100
2-ethylhexanol	GC	50

B. Non-volatile Residue Analysis

The nonvolatile residue from the extracts will be qualitatively examined for inorganic and organic substances. For inorganic species, ICP/MS and EDX/WDX will be employed. For non-volatile organic substances Infrared Spectroscopy will be employed.

An aliquot of each appropriate extract (10-20 mL) will be transferred to a suitable weighing dish and evaporated to dryness using a hot water bath. Other drying methods can be used but care should be taken to not degrade the residue.

Note that the choice of extracts submitted to these analyses will be made in consultation with all participating laboratories and investigators.

1. ICP/MS or EDX/WDX

For ICP, samples must be digested to obtain a solution as required in the referenced analytical method.[6] Digestions should be performed using aqueous solutions (i.e., aqueous solution of nitric acid).

For EDX/WDX the dried residues of the extracts are mounted for analysis. A scanning electron microprobe or other suitable analytical instrument is used to generate the x-ray spectrum showing the elements detected in the sample. The results are reported qualitatively.

2. Infrared Spectroscopic Analysis

The residue from the extract can be transferred onto a KBr or KRS-5 crystal with the aid of a solvent if necessary. The sample should be scanned 100X from 4000-400cm^{-1} having resolution of at least four cm^{-1}. The spectra can qualitatively evaluated by comparing to a spectral library or identification of major functional groups.

C. GC/MS (Gas Chromatography/Mass Spectrometry)

Semi-volatile compounds will be analyzed by Gas Chromatography/Mass Spectrometry (GC-MS) using a predominantly non-polar capillary column with wide (40 °C to 300 °C) temperature programming.[7] Each GC/MS analysis will produce an extractables "profile" in the form of a Total Ion Chromatogram (TIC). As a first pass, identifications of individual extractables will be accomplished with manual interpretation of the EI spectra (Electron Ionization) assisted by computerized mass spectral library searching. Beyond this, more difficult identifications may require the collection of additional data (such as Chemical Ionization GC/MS for molecular weight confirmation and High Resolution Mass Spectrometry for elemental composition), the purchase of reference compounds, *etc.*

The following GC/MS conditions are provided as an example. Any non-polar (100% dimethyl siloxane) or slightly polar (5% diphenyl siloxane) column can be used along with full temperature programming. Data cannot be collected while the injection solvent is in the ion source.

Note that additional identification work beyond the first pass analysis will be accomplished only after consultation with all participating laboratories and investigators.

Also note that the GC/MS instrumental conditions presented below are target conditions for all participating laboratories and investigators. The actual conditions employed by any participating laboratory should be reviewed by the entire group of participating investigators so that harmonization between laboratories can be preserved.

Gas Chromatograph Conditions	
Instrument:	Hewlett-Packard 5890 Series II Plus, Agilent 6890, or equivalent
Injection Mode:	cool on-column or splitless injection
Injection Volume:	1 µL
Injector Temperature/Program:	40 °C initial; oven track ON for on-column injection 280 °C for splitless injection
Purge Valve:	On at 1.00 min, off initially
Column:	Restek Rtx-1, 30 m x 0.25 mm (0.1 µm film), or equivalent
Oven Temperature:	40 °C for 1 min, heated at 10 °C/min to 300 °C and hold for 10 min
Pressure Program:	Constant flow (helium) at 1 mL/min
Transfer Line:	280 °C

Mass Spectrometer Conditions	
Instrument:	Hewlett-Packard 5972, Agilent

	5973 MSD, or equivalent
Ionization Mode:	EI (electron ionization)
Scan Mode:	Scanning; m/z 50-650
Scan Cycle Time:	Approximately 2 seconds/scan

D. HPLC/DAD (High Performance Liquid Chromatography/Diode Array Detection)

UV active species will be identified in the extracts by retention time and UV spectral matches. Reverse phase HPLC conditions will be employed using a gradient range from 50% to 100% solvent.8 The chromatogram of the extracts will be compared to that of a library of compounds and identification confirmed by obtaining the actual compound and analyzing with the sample.

Note that the HPLC/DAD instrumental conditions presented below are target conditions for all participating laboratories and investigators. The actual conditions (i.e., solvent strength, etc.) employed by any participating laboratory should be reviewed by the entire group of participating investigators so that harmonization between laboratories can be preserved.

Liquid Chromatograph Conditions	
Instrument:	Hewlett-Packard 1050, Agilent 1100, or equivalent
Flow Rate:	1 mL/min
Injection Volume:	10 µL
UV Wavelength:	200 nm
Column:	Vydac (201tp5415) C18, 5µ particles 15 cm x 4.6 mm, or equivalent

Temperature	60 °C
Mobile Phase:	Initial 50:50 acetonitrile/water 11 minute linear gradient Final 100% acetonitrile Hold 8 min 50:50 ACN/water at 1.5 ml/min for 5 minutes at 25 minutes return to 1.0 mL/min

E. LC/MS (Liquid Chromatography/Mass Spectrometry)

Compounds will be analyzed by Liquid Chromatography/Mass Spectrometry with in-line ultraviolet absorbance detection (LC/MS). The method will use reversed-phase chromatography with a wide (gradient) range of solvent strengths.9 Each LC/MS analysis will produce two extractables "profiles" in the form of a Total Ion Chromatogram (TIC) and a UV chromatogram. As a first pass, identifications of individual extractables will be accomplished with manual interpretation of the Atmospheric Pressure Chemical Ionization (APCI) spectra. Note that computerized mass spectral library searching is not available for APCI. Correlation with the GC/MS profiles will be attempted manually.

Beyond this, more difficult identifications may require the collection of additional data such as tandem mass spectrometry (MS/MS) for induced fragmentation, the purchase of reference compounds, *etc.*

Note that additional identification work beyond the first pass analysis will be accomplished only after consultation with all participating laboratories and investigators.

Also note that the LC/MS instrumental conditions presented below are target conditions for all participating laboratories and investigators. The actual conditions (i.e., solvent strength, etc.) employed by any participating laboratory should be reviewed by the entire group of participating investigators so that harmonization between laboratories can be preserved.

Liquid Chromatograph Conditions	
Instrument:	Hewlett-Packard 1050, Agilent 1100, or equivalent
Injection Volume:	10-50 µL, as appropriate
UV Wavelength:	280 nm

Column:	Alltech Alltima C18, 4.6 mm x 25 cm 5 µm particles, or equivalent
Mobile Phase:	A – 75:25 acetonitrile/water B – 50:50 acetonitrile/tetrahydrofuran

5122
5123

Gradient:		
Time (minutes)	**% A**	**% B**
0	100	0
10	60	40
20	0	100
30	0	100
32	100	0
45	100	0

5124
5125

Mass Spectrometer Conditions	
Instrument:	Micromass Platform II, Agilent 1100 MSD, or equivalent
Ionization Mode:	APCI (Atmospheric Pressure Chemical Ionization) (both APCI+ and APCI- will be accomplished)
Scan Mode:	Scanning; m/z 50-1350
Scan Cycle Time:	Approximately 5 seconds/scan

5126
5127

5128 IX. ANALYTICAL PROCEDURES

5129 A. Qualitative Analysis Procedure

5130 1. Sample Extract Preparation

The resulting extracts will usually contain low-level amounts of extractables. Sample concentration may be necessary as well as solvent switching to provide compatible samples for different analytical instrumentation. It is possible to manipulate extracts to provide very large concentration ratios, but this also has the effect of concentrating normal solvent impurities. For known targets in well-characterized matrices this is possible. As this protocol is for characterization purposes, no analyte or matrix behavior will be presupposed. Therefore, extracts will be concentrated no more than 100x as can be considered reasonable given normal ACS reagent purities of 99+%.

Concentration may be affected by residue formation and reconstitution in a smaller volume or by concentration to a fixed volume. Solvents may be switched during these procedures as appropriate. Residues may be prepared using standard techniques, rotary evaporation, nitrogen blow-down, lyophilization or centrifugal evaporation. Details of the sample preparation techniques will be based on good scientific reasoning and recorded in the laboratory notebook at time of analysis.

Note that the actual conditions employed by any participating laboratory should be reviewed by the entire group of participating investigators so that harmonization between laboratories can be preserved.

2. Blank Solvent Extract Preparation

The solvent blanks are extracted and prepared in the same manner as the sample and analyzed prior to sample extracts

3. Analysis

The extracts are surveyed using appropriate analytical methodology described in section VIII.

B. Quantitative Analysis Procedure (if required)

1. Sample Extract Preparation

The sample extracts can be obtain from the qualitative solutions or new extracts can be prepared to optimize for the extraction and analysis techniques.

2. Blank Solvent Extract Preparation

A blank solvent extract is prepared in the same manner as the sample and analyzed prior to sample analysis.

3. Standard Reference Material Preparation

Standard reference materials can be prepared at the appropriate concentrations as mixtures in a single solvent. Quantitative standardization will be performed using a single point relative to an internal or external standard.

4. Analysis

The extracts will be analyzed using methods that are optimized to detect the substances identified in the survey analysis.

Note that the actual conditions and procedures employed by any participating laboratory should be reviewed by the entire group of participating investigators so that harmonization between laboratories can be preserved.

X. DATA EVALUATION AND REPORTING

A. Qualitative Analysis

- A list of all identified extractables for all techniques will be generated that were not detected in the corresponding blank
- A list of all unidentified peaks in chromatogram that were not detected in the corresponding blank at signal to noise ratios greater than 10
- Amount of nonvolatile residue relative toward blank
- Indication of presence of known materials and techniques used in detection
- For each extraction, the solvents, condition and sample size
- For each analytical technique, the equipment, conditions and calibration method
- Provide copies of chromatograms and spectra

B. Quantitative Measurement (if required)

- List of analytes and source of standard reference materials
- Extraction and analysis techniques needed to determine all analytes
- For each extraction, the solvents, condition and sample size
- For each analytical technique, the equipment, conditions and calibration method
- Report as μg/gram sample
- Comparison to the known analytes/amounts
- Provide copies of sample and standard reference chromatograms and spectra

XI. GLOSSARY

ABBREVIATIONS

GC/FID	Gas Chromatograph Flame Ionization Detector
OVI	Organic Volatile Impurities
EDX	Energy Dispersive X-ray
WDX	Wavelength Dispersive X-ray
ICP/MS	Inductively Coupled Plasma Mass Spectrometer
GC/MS	Gas Chromatography Mass Spectrometry
HPLC/DAD	High Pressure Liquid Chromatography-Diode Array Detection
LC/MS	Liquid Chromatography Mass Spectrometry
AES	Atomic Emission Spectroscopy
ELSD	Evaporative Light Scattering Detector
RI	Refractive Index
TIC	Total Ion Chromatogram
APCI	Atmospheric Pressure Chemical Ionization

COMPOUNDS	**CAS NUMBERS**
Pyrene	129-00-0
2-Mercaptobenzothiazole	149-30-4
Tetramethylthiuramdisulfide	137-26-8
Butylatedhydroxytoluene (BHT)	128-37-0
Diphenylamine	122-37-4
Bis (2-ethylhexyl) phthalate	117-81-7
Bis (dodecyl) phthalate	2432-90-8
Stearic acid	57-11-4
2-ethylhexanol	104-76-7

XII. REFERENCES

[1] *Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products* Chemistry, Manufacturing, and Controls Documentation, CDER/FDA, October 1998, (Docket No. 98D-0997), available at http://www.fda.gov/cder/guidance/2180.pdf.

[2] *Guidance for Industry, Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products Chemistry, Manufacturing, and Controls Documentation*, CDER/FDA, July 2002, is available at http://www.fda.gov/cder/guidance/4234fnl.pdf

[3] PQRI Leachables and Extractables Working Group Work Plan: *Reporting and Qualification Thresholds for Leachables in Orally Inhaled and Nasal Drug Products*, (July 2002)

[4] Code of Federal Regulations: 21 CFR 210 and 211

[5] These experiments are considered research projects to be conducted in research labs, which are not strictly GMP compliant. However, all participating labs will perform these experiments in the spirit of GMP, which means that they will implement appropriate documentation, sample handling, data traceability, *etc.*

[6] Magellan Analytical Test Method ATM-MAG-M0012 "Determination of Metals by Inductively Coupled Plasma Mass Spectrometry"

[7] D.L. Norwood, D. Prime, B. Downey, J. Creasey, S. Sethi and P. Haywood, "Analysis of polycyclic aromatic hydrocarbons in metered dose inhaler drug formulations by isotope dilution gas chromatography/mass spectrometry," *J. Pharm. Biomed. Anal.*, 13(3), pp. 293-304 (1995)

[8] ASTM Designation: D 5524-94 "Standard Test Method for Determination of Phenolic Antioxidants in High Density Polyethylene Using Liquid Chromatography"

[9] J.D. Vargo and K.L. Olson, "Characterization of Additives in Plastics by Liquid Chromatography-Mass Spectrometry," *J. Chrom.*, 353, pp. 215-224 (1986)

Leachables and Extractables Working Group

21 January 2003

Experimental Protocol for Controlled Extraction Studies on Plastic Test Articles

Submitted to DPTC on 19 November 2002
Approved by DPTC January 2003

TABLE OF CONTENTS

I. INTRODUCTION

In November 1998 and May 1999, the FDA issued two CMC draft Guidances addressing Orally Inhaled and Nasal Drug Products (OINDP): (i) the draft *Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products Chemistry, Manufacturing, and Controls Documentation*[1] (referred to here as the "MDI/DPI draft Guidance"); and (ii) the draft *Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products Chemistry, Manufacturing, and Controls Documentation* (referred to here as the "Nasal Spray draft Guidance"). In July 2002, the Nasal Spray Guidance was finalized.[2]

Currently, both Guidances recommend that the Sponsor identify, report, and conduct toxicological analyses on all extractables found in the controlled extraction study (referred to in the Guidances as a "control extraction study"). Examples of these recommendations are described in the draft MDI/DPI Guidance regarding MDI canisters, valves, and actuators (lines 883-884; 990-991; and 1073):

> *…the profile of each extract should be evaluated both analytically and toxicologically.*

The Product Quality Research Institute (PQRI) Leachables and Extractables Working Group has developed this experimental protocol as an example of a Controlled Extraction Study for plastic test articles. Various experimental parameters will be investigated, test article extracts analyzed and results evaluated within the context of the Working Group's approved Work Plan and experimental hypothesis.

This experimental protocol will be used by all laboratories and investigators participating in the study.

II. PURPOSE AND SCOPE OF WORK

A. Purpose

The purpose of the experiments outlined in this protocol is to generate data from Controlled Extraction Studies that will contribute to a larger database, which the Working Group will use to investigate its hypotheses:[3]

1. Scientifically justifiable thresholds based on the best available data and industry practices can be developed for:

   (a) the reporting and safety qualification of leachables in orally inhaled and nasal drug products, and

   (b) reporting of extractables from the critical components used in corresponding container/closure systems.

   Reporting thresholds for leachables and extractables will include associated identification and quantitation thresholds.

2. Safety qualification of extractables would be scientifically justified on a case-by-case basis.

B. Scope

1. Topics Addressed by This Protocol

This protocol covers only Controlled Extraction Studies that would be applied to components from Metered Dose Inhalers (MDIs). The MDI represents the best example of "correlation" between extractables from components and leachables in drug product. Controlled Extraction Studies will be performed following the general outline described in the Guidances. Test articles will be subjected to different extraction conditions to show how different experimentally controlled parameters affect resulting extractables profiles. Additionally, the Working Group will assess experimental results to identify reasonable approaches for sample preparation and analysis of extractables from container and closure components.

As no single analytical technique can be used to identify and quantify all unknown extractables, a variety of methods will be utilized in this protocol to maximize the likelihood that all extractable compounds associated with the test articles are evaluated analytically. Overlap between methods will supply corroborating data that the procedures are valid. To provide a full analytical survey of possible analytes the following strategy will be employed:

2. Direct injection Gas Chromatography/Mass Spectrometry (GC/MS) for identification and assessment of relatively volatile extractables.

3. High Performance Liquid Chromatography/Diode Array Detection (HPLC/DAD) for identification and assessment of relatively polar/non-volatile UV active extractables.

4. High Performance Liquid Chromatography/Mass Spectrometry (LC/MS) for identification and assessment of relatively polar/non-volatile extractables, which may or may not have UV activity.

5. Inductively Coupled Plasma/Mass Spectrometry (ICP/MS), Inductively Coupled Plasma/Atomic Emission Spectroscopy (ICP/AES), or Energy Dispersive X-ray (EDX) /Wavelength Dispersive X-ray (WDX) to detect single elements in the extracts (i.e., metals).

6. Fourier Transform Infrared Spectroscopy (FTIR) for characterization of major components in the non-volatile extractable residues.

2. Topics Not Addressed by This Protocol

Studies designed to assess recovery (i.e., mass balance) for individual extractables relative to the known formulations of chemical additives in the plastic test articles, or

reproducibility of extractables profiles for multiple "batches" of any particular test article are not within the scope of this test protocol.

The extraction procedures, analytical techniques/methods, and analysis conditions described in this experimental test protocol will not be validated as material control methods, since they will be performed in order to collect qualitative information. However, during the course of these experiments the PQRI Leachables and Extractables Working Group will review the results and may initiate additional experimental work for quantitative assessment of extractables.

This protocol does not address system suitability tests for quantitative methods. Appropriate system suitability tests will be addressed later and agreement on this issue will be reached with all of the participating laboratories.

Special case studies such as OVIs (Organic Volatile Impurities), N-nitrosamines or Polynuclear Aromatic Hydrocarbons (PAHs or PNAs) will not be considered in this study. These "special case" classes of extractables have defined and highly specific analytical methods which are generally accepted and commonly used for their identification and quantitative assessment.

It should be noted that the outlined experimental procedures, analytical instrumentation parameters and conditions, and other details are intended as a guideline for laboratory studies. Details of actual experimental procedures, etc., should be reviewed by the entire group of participating laboratories and investigators so that harmonization between laboratories working on the same test articles can be achieved.

III. REGULATORY STATUS

This is a Good Manufacturing Practices (GMP)[4] study. All experiments shall be performed under GMP conditions to the extent practical in a particular laboratory.[5] Any changes to these protocols shall be documented, following appropriate GMP change control procedures.

IV. SAFETY AND ENVIRONMENTAL IMPACT

Organic solvents are commonly used to enhance solubility of lipophilic targets and to increase transport of small molecules out of complex matrices. These solvents may be flammable and/or show short-term and long-term environmental health risks. Care must be exercised with their use. Consult the Material Safety and Data Sheet (MSDS) for appropriate personal protection and disposal.

V. TEST ARTICLES

Polypropylene and Low Density Polyethylene (LDPE) materials will be provided in disc form for use as test articles. The additive formulations and manufacturing conditions for these test articles are known and will be provided to all laboratories participating in the study.

The following known formulation ingredients will be provided for use as identification and potentially quantitation reference compounds/mixtures:

- Irganox 1010
- Ultranox 626
- Calcium Stearate
- Pationic 901
- Millad 3988

Note that additional reference compounds and additive mixtures may be required for the completion of this test protocol and will be provided as appropriate.

VI. CHEMICALS AND EQUIPMENT

Extraction and analytical methods have been chosen and designed so as to utilize chemicals, apparatus, and instrumentation available in typical laboratories routinely involved with this type of study.

A. Extraction Solvents

Extractions will be performed on each test article using three solvents representing a range of polarity selected from the list below. The solvents should be American Chemical Society (ACS) grade or better:

- methylene chloride (dichloromethane)
- 2-propanol (isopropanol)
- hexane (n-hexane, not hexanes)

Depending on the behavior of the test articles in these particular solvent systems, additional solvents may be chosen. Changes in extracting solvent will be discussed by all study participants prior to change initiation by any particular study participant or laboratory.

B. Extraction Apparatus

- Soxhlet apparatus with an Allhin condenser, flask (500 mL), and hot plate or heating mantle
- Sonicator
- Reflux apparatus consisting of an Erlenmeyer flask (125 mL or larger) and condenser with ground glass joints, hot plate or heating mantle.

C. Analytical Instrumentation

- Gas chromatograph equipped with a Flame Ionization Detector (GC/FID)
- Gas chromatograph equipped with a Mass Spectrometer (GC/MS)
- Liquid chromatograph equipped with a photodiode array detector
- Liquid chromatograph equipped with an APCI (Atmospheric Pressure Chemical Ionization) capable Mass Spectrometer (LC/MS)
- Fourier Transform Infrared spectrometer (FTIR)
- EDX and/or WDX equipped with a microprobe or scanning electron microprobe
- Inductively Coupled Plasma Mass Spectrometry (ICP/MS) and/or Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP/AES)

VII. EXTRACTION PROCEDURES

For each extraction technique and solvent type, appropriate blanks (no test article sample) must be prepared. These must be prepared concurrently using a different extraction apparatus (same type) under the same conditions, or by using the same apparatus prior to charging with sample.

Note that the extraction parameters and conditions outlined below are subject to modification and the details of any particular extraction process must be agreed to between all laboratory study participants prior to initiation of experimental work in any particular laboratory.

A. Soxhlet Extraction

1. Sample Preparation

Samples of each test article should be cut into strips appropriately sized to fit into pre-extracted Soxhlet cellulose thimbles. Sample amounts may be in the range of 1-3 g (2 g) using 200 mL of solvent. For quantitative measurements, extracts prepared by Soxhlet will have to be evaporated to dryness and the resulting residues re-dissolved to a known volume (25-50 mL). Alternatively an internal standard can be used for quantitative measurements.

2. Extraction Conditions

Under normal laboratory conditions, three physical extraction parameters may be modified, turnover number, total extraction time and temperature. Temperature is the most difficult of the three parameters to control as the sample holder is maintained above the vapor level (temperature may be above the boiling point), but will be continuously

bathed in freshly distilled solvent (coil temperature). It is recommended that the coil temperature be kept as low as possible to avoid heating above the solvent flashpoint.

Turnover number is controlled by the heating rate and should be limited by safety concerns. At low turnover numbers, the extraction characteristics will resemble those of reflux and may be limited by equilibrium phenomena. It is recommended that turnover numbers to be at least ten during the course of the extraction.

Extraction time should be in the range of 24 hours to guard against possible degradation of thermally labile or reactive compounds.

B. Reflux

Reflux extraction is a common and easily implemented approach for the production of extractables (e.g., USP <381> "Elastomeric Closures-Physicochemical Tests"). Conditions are easily standardized as the temperature and pressure are at the defined boiling points of the extraction solvents. Unlike Soxhlet extraction, reflux extraction is an equilibrium phenomenon.

1. Sample Preparation

Transport of extractables out of the complex matrix may be affected by the surface area and thickness of the test article. Test articles will be prepared by three methods: pressing, grinding, and cutting into strips appropriately sized to fit into the reflux apparatus.

Sample amounts should be in the range of 2 g using 25–50 mL of solvent. For quantitative measurements the solvent with sample and flask can be weighed and returned to original weight after extraction. Alternatively an internal standard can be used for quantitative measurements.

In reflux extraction, the sample to solvent ratio may affect the completeness of the technique. This should be addressed when optimizing the method for measurement of extractables

2. Extraction Conditions

The only adjustable physical parameter for reflux extraction is time. Extraction time should be 2 to 4 hours. The solvent reservoir level must be monitored and periodically recharged to provide the correct amount of solvent.

C. Sonication

Sonication uses ultrasonic energy instead of thermal energy to increase the rate of diffusion of small analytes out of a solid matrix. Similar considerations as reflux extraction (equilibrium conditions) should be evaluated, but these cannot be calculated using thermodynamic parameters. Sonication equipment may be standardized by measuring the temperature rise after a set exposure time and evaluating the energy

deposited into the solvent. Standardization of conditions should be accomplished after consultation between participating laboratories.

1. Sample Preparation

Transport of extractables out of the complex matrix may be affected by surface area and thickness of the test article. Test articles will be prepared by three methods: pressing, grinding, and cutting into strips appropriately sized to fit into the sonication apparatus.

In sonication, the sample to solvent ratio may affect the completeness of the technique. Therefore, a weight ratio of at least 20:1 solvent to sample should be maintained with sample amounts of 2 g.

2. Extraction Conditions

The only adjustable physical parameter for sonication is time. Bath temperatures should be standardized using either ice-water (0 °C), or monitored by a calibrated thermometer. Extractions may be completed in as little as 15 minutes. Safety considerations are paramount as extractions are performed under normal atmosphere and the technique may provide easy ignition. The solvent reservoir level must be periodically recharged to provide the correct amount of solvent.

VIII. ANALYTICAL METHODS

A. Chromatographic Methods System Suitability for Extractables Profiling (Qualitative Analyses)

Standard reference materials will be used for qualitative chromatographic analytical techniques to ensure system suitability. The standard reference materials are selected to represent a range of common extractable compounds found in polymeric materials. No one analytical technique is suitable for detection of all targets. The following table presents a list of system suitability analytes for GC and HPLC based analytical techniques. The presence of these analytes should be verified at the recommended concentrations prior to analysis of test article extracts by any participating laboratory.

Note that the entire group of participating laboratories and scientists will judge whether a given participating laboratory has met system suitability for its analytical techniques prior to that laboratory analyzing test article extracts.

Compound Name	Suggested Techniques	Recommended Target Concentration (µg/mL)
Pyrene	GC and LC/UV	1 ppm
2-Mercaptobenzothiazole	GC or LC	50 ppm
Tetramethylthiuramdisulfide	GC and LC/UV	50 ppm
Butylatedhydroxytoluene	GC or LC	50 ppm

(BHT)		
Irganox 1010	LC	50 ppm
Diphenylamine	LC	50 ppm
Bis (2-ethylhexyl) phthalate	GC or LC	50 ppm
Bis (dodecyl) phthalate	GC or LC	50 ppm
Stearic acid	GC and LC/MS	100 ppm
2-ethylhexanol	GC	50 ppm

B. Non-volatile Residue Analysis

The nonvolatile residue from the extracts will be qualitatively examined for inorganic and organic substances. For inorganic species, ICP/MS and EDX/WDX will be employed. For non-volatile organic substances Infrared Spectroscopy will be employed.

An aliquot of each appropriate extract (10-20 mL) will be transferred to a suitable weighing dish and evaporated to dryness using a hot water bath. Other drying methods can be used but care should be taken to not degrade the residue.

Note that the choice of extracts submitted to these analyses will be made in consultation with all participating laboratories and investigators.

1. ICP/MS or EDX/WDX

For ICP, samples must be digested to obtain a solution as required in the referenced analytical method.[6] Digestions should be performed using aqueous solutions (i.e., aqueous solution of nitric acid).

For EDX/WDX the dried residues of the extracts are mounted for analysis. A scanning electron microprobe or other suitable analytical instrument is used to generate the x-ray spectrum showing the elements detected in the sample. The results are reported qualitatively.

2. Infrared Spectroscopic Analysis

The residue from the extract can be transferred onto a KBr or KRS-5 crystal with the aid of a solvent if necessary. The sample should be scanned 100X from 4000-400cm^{-1} having resolution of at least four cm^{-1}. The spectra can qualitatively evaluated by comparing to a spectral library or identification of major functional groups.

C. GC/MS (Gas Chromatography/Mass Spectrometry)

Semi-volatile compounds will be analyzed by Gas Chromatography/Mass Spectrometry (GC-MS) using a predominantly non-polar capillary column with wide (40 °C to 300 °C) temperature programming.[7] Each GC/MS analysis will produce an extractables “profile”

in the form of a Total Ion Chromatogram (TIC). As a first pass, identifications of individual extractables will be accomplished with manual interpretation of the EI spectra (Electron Ionization) assisted by computerized mass spectral library searching. Beyond this, more difficult identifications may require the collection of additional data (such as Chemical Ionization GC/MS for molecular weight confirmation and High Resolution Mass Spectrometry for elemental composition), the purchase of reference compounds, *etc*.

The following GC/MS conditions are provided as an example. Any non-polar (100% dimethyl siloxane) or slightly polar (5% diphenyl siloxane) column can be used along with full temperature programming. Data cannot be collected while the injection solvent is in the ion source.

Note that additional identification work beyond the first pass analysis will be accomplished only after consultation with all participating laboratories and investigators.

Also note that the GC/MS instrumental conditions presented below are target conditions for all participating laboratories and investigators. The actual conditions employed by any participating laboratory should be reviewed by the entire group of participating investigators so that harmonization between laboratories can be preserved.

Gas Chromatograph Conditions	
Instrument:	Hewlett-Packard 5890 Series II Plus, Agilent 6890, or equivalent
Injection Mode:	Cool on-column or splitless injection
Injection Volume:	1 µL
Injector Temperature/Program:	40 °C initial; oven track ON for on-column injection 280 °C for splitless injection
Purge Valve:	On at 1.00 min, off initially
Column:	Restek Rtx-1, 30 m x 0.25 mm (0.1 µm film) or equivalent
	40 °C for 1 min, heated at

Oven Temperature:	10 °C/min to 300 °C and hold for 10 min
Pressure Program:	Constant flow (helium) at 1 mL/min
Transfer Line:	280 °C

Mass Spectrometer Conditions	
Instrument:	Hewlett-Packard 5972, Agilent 5973 MSD , or equivalent
Ionization Mode:	EI (electron ionization)
Scan Mode:	Scanning; m/z 50-650
Scan Cycle Time:	Approximately 2 seconds/scan

D. HPLC/DAD (High Performance Liquid Chromatography/Diode Array Detection)

UV active species will be identified in the extracts by retention time and UV spectral matches. Reverse phase HPLC conditions will be employed using a gradient range from 50% to 100% solvent.[8] The chromatogram of the extracts will be compared to that of a library of compounds and identification confirmed by obtaining the actual compound and analyzing with the sample.

Note that the HPLC/DAD instrumental conditions presented below are target conditions for all participating laboratories and investigators. The actual conditions (i.e., solvent strength, etc.) employed by any participating laboratory should be reviewed by the entire group of participating investigators so that harmonization between laboratories can be preserved.

Liquid Chromatograph Conditions	
Instrument:	Hewlett-Packard 1050, Agilent 1100 or

	equivalent
Flow Rate:	1 mL/min
Injection Volume:	10 µL
UV Wavelength:	200 nm
Column:	Vydac (201tp5415) C18, 5µ particles 15 cm x 4.6 mm, or equivalent
Temperature	60 °C
Mobile Phase:	Initial 50:50 acetonitrile/water 11 minute linear gradient Final 100% acetonitrile Hold 8 min 50:50 ACN/water at 1.5 mL/min for 5 minutes at 25 minutes return to 1.0 mL/min

E. LC/MS (Liquid Chromatography/Mass Spectrometry)

Compounds will be analyzed by Liquid Chromatography/Mass Spectrometry with in-line ultraviolet absorbance detection (LC/MS). The method will use reversed-phase chromatography with a wide (gradient) range of solvent strengths.[9] Each LC/MS analysis will produce two extractables "profiles" in the form of a Total Ion Chromatogram (TIC) and a UV chromatogram. As a first pass, identifications of individual extractables will be accomplished with manual interpretation of the Atmospheric Pressure Chemical Ionization (APCI) spectra. Note that computerized mass spectral library searching is not available for APCI. Correlation with the GC/MS profiles will be attempted manually.

Beyond this, more difficult identifications may require the collection of additional data such as tandem mass spectrometry (MS/MS) for induced fragmentation, the purchase of reference compounds, *etc.*

Note that additional identification work beyond the first pass analysis will be accomplished only after consultation with all participating laboratories and investigators.

Also note that the LC/MS instrumental conditions presented below are target conditions for all participating laboratories and investigators. The actual conditions (i.e., solvent strength, etc.) employed by any participating laboratory should be reviewed by the entire group of participating investigators so that harmonization between laboratories can be preserved.

Liquid Chromatograph Conditions	
Instrument:	Hewlett-Packard 1050, Agilent 1100, or equivalent
Injection Volume:	10-50 µL, as appropriate
UV Wavelength:	280 nm
Column:	Alltech Alltima C18, 4.6 mm x 25 cm 5 µm particles, or equivalent
Mobile Phase:	A – 75:25 acetonitrile/water B – 50:50 acetonitrile/tetrahydrofuran

Gradient:

Time (minutes)	**% A**	**% B**
0	100	0
10	60	40
20	0	100
30	0	100
32	100	0
45	100	0

Mass Spectrometer Conditions	
Instrument:	Micromass Platform II, Agilent 1100 MSD, or equivalent

Ionization Mode:	APCI (Atmospheric Pressure Chemical Ionization) (both APCI+ and APCI- will be accomplished)
Scan Mode:	Scanning; m/z 50-1350
Scan Cycle Time:	Approximately 5 seconds/scan

IX. ANALYTICAL PROCEDURES

A. Qualitative Analysis Procedure

1. Sample Extract Preparation

The resulting extracts will usually contain low-level amounts of extractables. Sample concentration may be necessary as well as solvent switching to provide compatible samples for different analytical instrumentation. It is possible to manipulate extracts to provide very large concentration ratios, but this also has the effect of concentrating normal solvent impurities. For known targets in well-characterized matrices this is possible. As this protocol is for characterization purposes, no analyte or matrix behavior will be presupposed. Therefore, extracts will be concentrated no more than 100x as can be considered reasonable given normal ACS reagent purities of 99+%.

Concentration may be affected by residue formation and reconstitution in a smaller volume or by concentration to a fixed volume. Solvents may be switched during these procedures as appropriate. Residues may be prepared using standard techniques, rotary evaporation, nitrogen blow-down, lyophilization or centrifugal evaporation. Details of the sample preparation techniques will be based on good scientific reasoning and recorded in the laboratory notebook at time of analysis.

Note that the actual conditions employed by any participating laboratory should be reviewed by the entire group of participating investigators so that harmonization between laboratories can be preserved.

2. Blank Solvent Extract Preparation

The solvent blanks are extracted and prepared in the same manner as the sample and analyzed prior to sample extracts

3. Analysis

The extracts are surveyed using appropriate analytical methodology described in section VIII.

B. Quantitative Analysis Procedure (if required)

1. Sample Extract Preparation

The sample extracts can be obtained from the qualitative solutions or new extracts can be prepared to optimize for the extraction and analysis techniques.

2. Blank Solvent Extract Preparation

A blank solvent extract is prepared in the same manner as the sample and analyzed prior to sample analysis.

3. Standard Reference Material Preparation

Standard reference materials can be prepared at the appropriate concentrations as mixtures in a single solvent. Quantitative standardization will be performed using a single point relative to an internal or external standard.

4. Analysis

The extracts will be analyzed using methods that are optimized to detect the substances identified in the survey analysis.

Note that the actual conditions and procedures employed by any participating laboratory should be reviewed by the entire group of participating investigators so that harmonization between laboratories can be preserved.

X. DATA EVALUATION AND REPORTING

A. Qualitative Analysis

- A list of all identified extractables for all techniques will be generated that were not detected in the corresponding blank
- A list of all unidentified peaks in chromatogram that were not detected in the corresponding blank at signal to noise ratios greater than 10
- Amount of nonvolatile residue relative toward blank
- Indication of presence of known materials and techniques used in detection
- For each extraction, the solvents, condition and sample size
- For each analytical technique the equipment, conditions and calibration method
- Provide copies of chromatograms and spectra

B. Quantitative Measurement (if required)

- List of analytes and source of standard reference materials
- Extraction and analysis techniques needed to determine all analytes

- For each extraction the solvents, condition and sample size
- For each analytical technique the equipment, conditions and calibration method
- Report as µg/gram sample
- Comparison to the known analytes/amounts
- Provide copies of sample and standard reference chromatograms and spectra

XI. GLOSSARY

ABBREVIATIONS

GC/FID	Gas Chromatograph Flame Ionization Detector
OVI	Organic Volatile Impurities
EDX	Energy Dispersive X-ray
WDX	Wavelength Dispersive X-ray
ICP/MS	Inductively Coupled Plasma Mass Spectrometer
GC/MS	Gas Chromatography Mass Spectrometry
HPLC/DAD	High Pressure Liquid Chromatography-Diode Array Detection
LC/MS	Liquid Chromatography Mass Spectrometry
AES	Atomic Emission Spectroscopy
ELSD	Evaporative Light Scattering Detector
RI	Refractive Index
TIC	Total Ion Chromatogram
APCI	Atmospheric Pressure Chemical Ionization

COMPOUNDS	**CAS NUMBERS**
Pyrene	129-00-0
2-Mercaptobenzothiazole	149-30-4
Tetramethylthiuramdisulfide	137-26-8
Butylatedhydroxytoluene (BHT)	128-37-0
Diphenylamine	122-39-4
Bis (2-ethylhexyl) phthalate	117-81-7
Bis (dodecyl) phthalate	2432-90-8

Stearic acid	57-11-4
2-ethylhexanol	104-76-7

5695 – References

[1] *Draft Guidance for Industry, Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products Chemistry, Manufacturing, and Controls Documentation*, CDER/FDA, October 1998, (Docket No. 98D-0997), available at http://www.fda.gov/cder/guidance/2180.pdf.

[2] *Guidance for Industry, Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products Chemistry, Manufacturing, and Controls Documentation*, CDER/FDA, July 2002, is available at http://www.fda.gov/cder/guidance/4234fnl.pdf

[3] PQRI Leachables and Extractables Working Group Work Plan: *Reporting and Qualification Thresholds for Leachables in Orally Inhaled and Nasal Drug Products*, (July 2002)

[4] Code of Federal Regulations: 21 CFR 210 and 211

[5] These experiments are considered research projects to be conducted in research labs, which are not strictly GMP compliant. However, all participating labs will perform these experiments in the spirit of GMP, which means that they will implement appropriate documentation, sample handling, data traceability, *etc.*

[6] Magellan Analytical Test Method ATM-MAG-M0012 "Determination of Metals by Inductively Coupled Plasma Mass Spectrometry"

[7] D.L. Norwood, D. Prime, B. Downey, J. Creasey, S. Sethi and P. Haywood, "Analysis of polycyclic aromatic hydrocarbons in metered dose inhaler drug formulations by isotope dilution gas chromatography/mass spectrometry," *J. Pharm. Biomed. Anal.*, 13(3), pp. 293-304 (1995)

[8] ASTM Designation: D 5524-94 "Standard Test Method for Determination of Phenolic Antioxidants in High Density Polyethylene Using Liquid Chromatography"

[9] J.D. Vargo and K.L. Olson, "Characterization of Additives in Plastics by Liquid Chromatography-Mass Spectrometry," *J. Chrom.*, 353, pp. 215-224 (1986)

PROTOCOL ADDITION

PHASE 2 STUDIES: QUANTITATIVE CONTROLLED EXTRACTION STUDIES ON THE SULFUR-CURED ELASTOMER

PQRI Leachables and Extractables Working Group

25 September 2003

TABLE OF CONTENTS

Protocol for Validation of a Quantitative Extractables Profiling Method for a Sulfur-Cured Elastomer Using Soxhlet Extraction And Gas Chromatography/Flame Ionization Detection

I. INTRODUCTION AND BACKGROUND

Qualitative Controlled Extraction studies guided by a specific and detailed protocol have been accomplished on a sulfur-cured elastomeric test article of known additive composition. These qualitative studies produced extractables profiles by GC/MS (Gas Chromatography/Mass Spectrometry) and LC/MS (High Performance Liquid Chromatography/Mass Spectrometry) which exactly reflect the known additive composition of the elastomeric test article.

This protocol addition is designed to extend the qualitative controlled extraction study to a quantitative controlled extraction study, with appropriate method optimization and investigation of validation parameters. The analytical system chosen for validation is GC/FID (Gas Chromatography/Flame Ionization Detection).

II. TEST ARTICLE

The elastomer test article to be employed in this study is a sulfur-cured and carbon black containing rubber especially created for this PQRI project by West Pharmaceutical Services. The qualitative extractables profile of this elastomeric material was fully characterized under a preceding test protocol.

III. METHOD DEVELOPMENT

Based on the results of the qualitative controlled extraction studies, Soxhlet extraction in methylene chloride with quantitative GC analysis of extracts has been selected for optimization and validation. Internal standardization utilizing appropriate authentic reference materials will be employed for quantitative calibration of the analytical system. The known additives in the elastomeric test article which can be quantitated by this analytical technique include:

- 2, 2´-methylene-bis(6-*tert*- butyl-4-ethyl phenol)
- Coumarone-Indene Resin related species
- n-alkanes derived from paraffin/oils
- additional relatively minor extractables

All details of the analytical method, including the extraction procedure and analysis system will be documented in laboratory notebooks and/or other appropriate documentation media.

Prior to method validation, the extraction procedure will be optimized to produce maximum quantities of target extractables (i.e., “asymptotic” levels; note the example experiment in Figure 4, page 12). The optimized extraction conditions will then be employed for an initial examination of extraction method repeatability. Individual representative target extractables will be used to evaluate linearity, various chromatographic parameters, establish appropriate dynamic ranges for quantitation, and assess method accuracy. The optimized quantitative analytical

method will then be taken to validation with acceptance criteria either based on the method development studies, or based on the expected performance of such analytical methods.

IV. VALIDATION PARAMETERS AND ACCEPTANCE CRITERIA

The following validation parameters which include appropriate acceptance criteria will be investigated. When appropriate, the following representative target extractables will be employed:

2, 2´-methylene-bis(6-*tert*- butyl-4-ethyl phenol)

n-Docosane

n-Tricosane

n-Tetracosane

n-Pentacosane

n-Hexacosane

n-Octacosane

Internal Standard: 2-fluorobiphenyl

These target extractables include the primary phenolic antioxidant and several n-alkanes which represent the bulk of the remaining extractables profile. The qualitative GC/MS extractables profile of the sulfur-cured elastomeric test article is shown in Figure 1 (see page 9), with extractables identifications in Table 1 (see page 13). A representative GC/FID extractables profile is shown in Figure 2 (see page 10).

A. System Suitability

1. Instrument Precision

A test solution of target extractables with internal standard will be prepared at concentrations demonstrated not to produce adverse effects on chromatographic performance, and at levels determined to encompass the concentrations of target extractables determined in the Method Development phase of this study. Utilizing optimized chromatography conditions, six (6) replicate injections of the test solution will be analyzed. Peak area and area ratio measurements of target extractables and the internal standard will be determined, and means and percent relative standard deviations (%RSDs) of area ratios and relative response factors will be calculated.

Acceptance Criteria: %RSDs for area ratios and relative response factors to be determined during method development

Note: Relative Response Factor (RRF) is defined as:

$$RRF = (A_a C_I)/(A_i C_a)$$

Where:

A_a = *area of analyte peak*

A_i = *area of internal standard peak*
C_a = *concentration of analyte*
C_i = *concentration of internal standard*

2. Chromatographic Resolution (USP)

Utilizing the analyses accomplished for Instrument Precision, chromatographic resolution between appropriate peak pairs will be determined. Means and percent relative standard deviations (%RSDs) will be calculated. Appropriate peak pairs will be selected during method development.

Acceptance Criteria: to be determined during method development

3. Chromatographic Tailing Factor (USP)

Utilizing the analyses accomplished for Instrument Precision, chromatographic tailing factors for appropriate peaks will be determined. Means and percent relative standard deviations (%RSDs) will be calculated. Appropriate peaks will be selected during method development.

Acceptance Criteria: to be determined during method development

B. Linearity and Range

Linearity and range will be determined by analyzing selected target extractables at six (6) different concentration levels (in duplicate), over a range established during the Method Development phase of this study. For each target extractable linearity experiment, a linear regression analysis will be accomplished on peak area ratios versus analyte concentration. Slopes, y-intercepts, and coefficients of determination (r^2) will be calculated.

Target extractables: 2, 2´-methylene-bis(6-*tert*- butyl-4-ethyl phenol)
Pentacosane

Acceptance Criteria: to be determined during method development

In addition to the linearity study for selected target extractables, single-point relative response factors will be determined for additional identified extractables for which authentic reference compounds are available. The list of extractables for which this will be accomplished and the concentration level at which the measurements will be made will be determined during the Method Development phase of the study. These additional extractables may or may not be limited to those listed in Table 1.

Acceptance Criteria: report results

C. Precision

1. Repeatability

Utilizing optimized extraction procedures, six (6) separate extractions will be accomplished and target extractables quantitated with the analytical method. Means and percent relative standard deviations (%RSDs) of individual target extractable amounts will be calculated.

Acceptance Criteria: %RSD for each target extractable ≤ 10%

2. Intermediate Precision

Intermediate Precision will be evaluated by a second analyst accomplishing the Repeatability study utilizing a different GC column, and analytical instrument (if available).

Acceptance Criteria:
1. *%RSD for each target extractable ≤ 10%*
2. *%difference between analyst means for each target extractable ≤ 25%*

D. Specificity

Specificity was demonstrated in the qualitative phase of the controlled extraction studies utilizing GC/MS (Gas Chromatography/Mass Spectrometry).

Acceptance Criteria: Confirms peak identifications and confirms no significant coeluting peaks for each target extractable.

E. Accuracy

Accuracy will be expressed as the percent recovery of known amounts of target extractables spiked into the extraction system.

Spiking solutions of appropriate target extractables will be prepared and spiked at three different levels (in triplicate). The individual spiking levels will be chosen to represent the appropriate range of analyte concentrations expected based on the method development experiments. Spiked samples will be analyzed by the optimized analytical method and individual mean recoveries determined for each spiking level.

Acceptance Criteria: Mean recovery for each target extractable at each spiking level should be between 80% and 120% of known spiking level.

F. Limit of Quantitation (LOQ)

A standard solution of target extractables designed to produce a response of approximately ten (10) times the LOQ, i.e., a response that provides a signal-to-noise (RMS) ratio (S/N) of approximately 100:1, will be analyzed six (6) times by the optimized analytical method. Based on the average signal-to-noise ratios for each target extractable, LOQs will be estimated by extrapolation (S/N 10:1). Based on these extrapolated LOQs, a solution of target extractables will be prepared and analyzed six (6) times for LOQ confirmation.

Acceptance Criteria: Report results based on extrapolated LOQs

G. Standard and Sample Stability

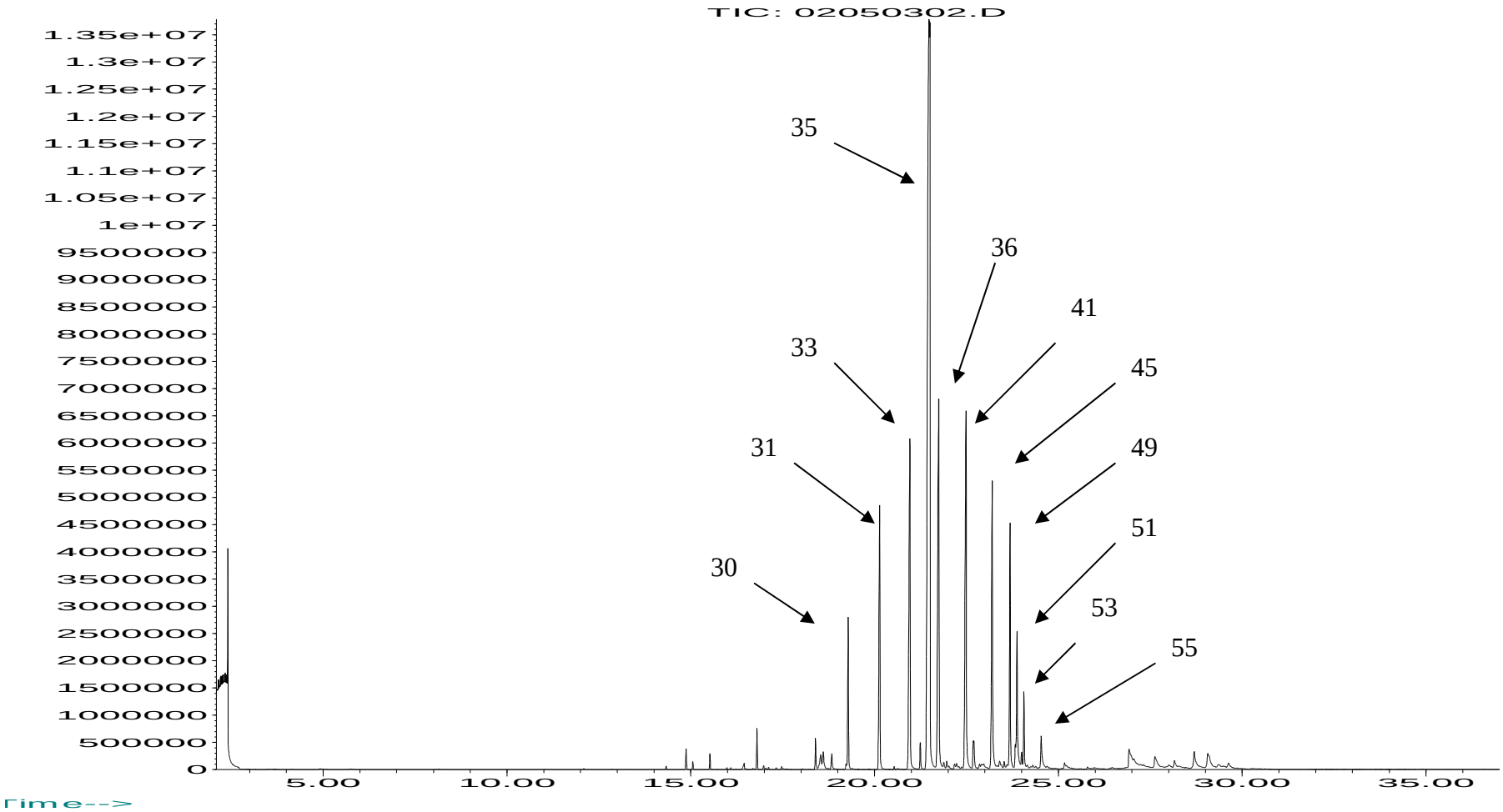
Standard and sample stability will be evaluated over a period of 5 days by analyzing on each day an appropriate mixed standard of target extractables (as in the System Suitability section), and an appropriate test article extract (as in the Precision section). Appropriate area ratios of target extractable to internal standard will be determined and the solutions will be considered stable if:

Acceptance Criteria: Area ratios for target extractables on each subsequent day should be ±10% of those determined on day 1.

H. Robustness/Ruggedness

Robustness/Ruggedness experiments will not be accomplished as a part of this validation protocol. However, this decision may be revisited and modified during the course of the validation exercise. Any robustness/ruggedness studies will be based on critical method parameters identified during the method development and validation phases of the study.

5881 Figure 1. GC/MS extractables profile (Total Ion Chromatogram; TIC) of the West sulfur-cured elastomer (16 hour Soxhlet
5882 extraction with dichloromethane).



5883 Figure 2. GC/MS extractables profile (Total Ion Chromatogram; TIC) of the West sulfur-cured elastomer (16 hour Soxhlet
5884 extraction with dichloromethane; internal standard added; optimized injection volume).

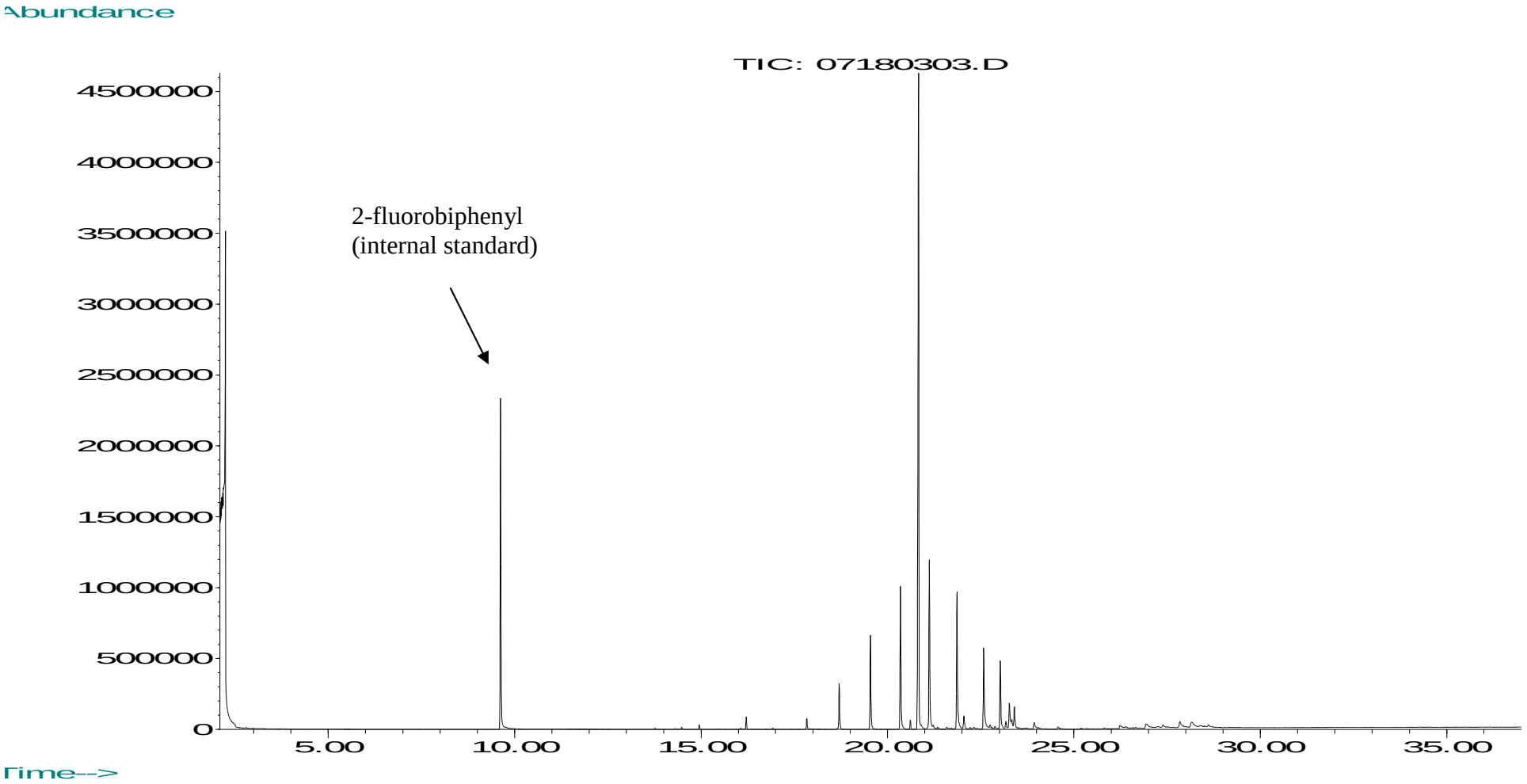
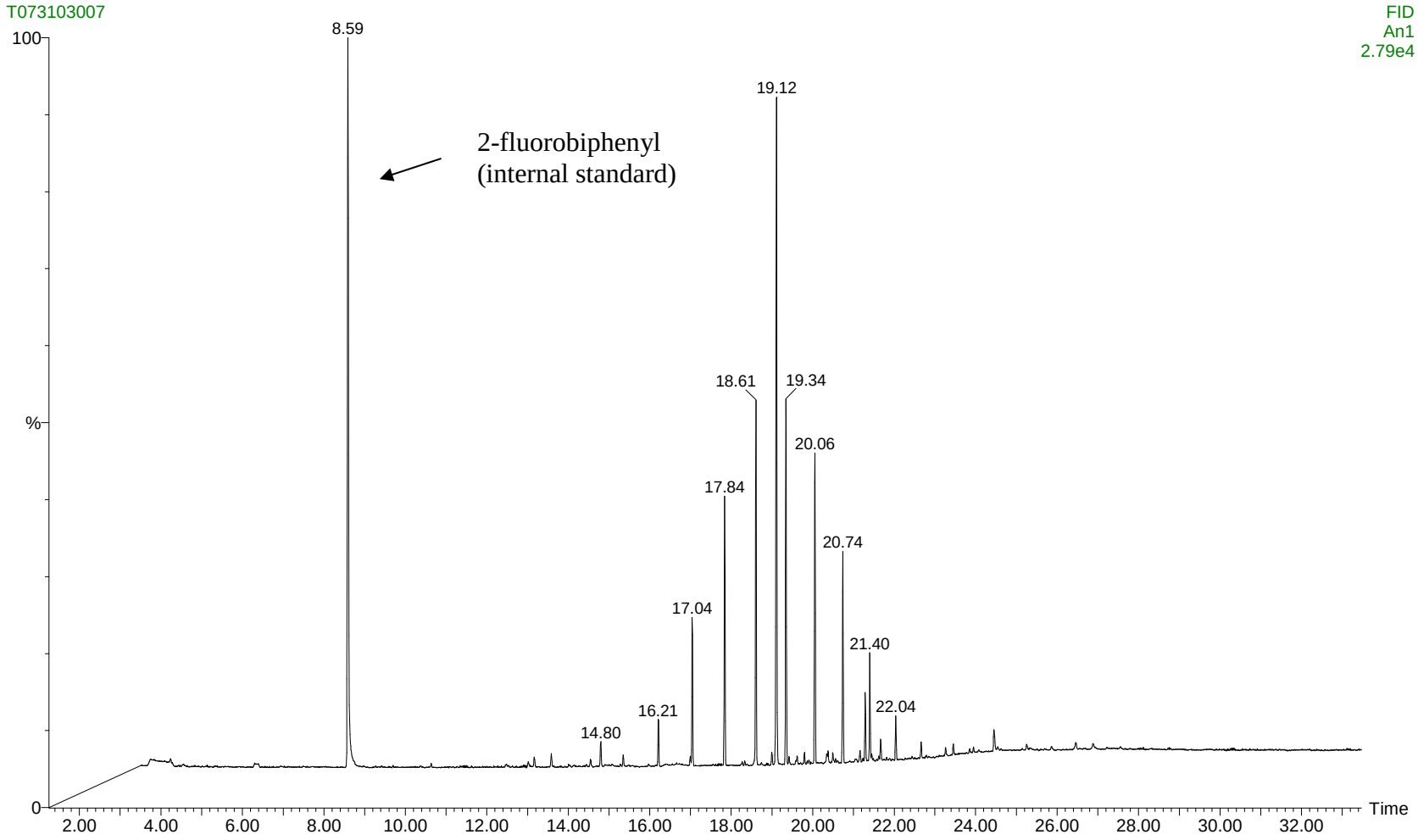
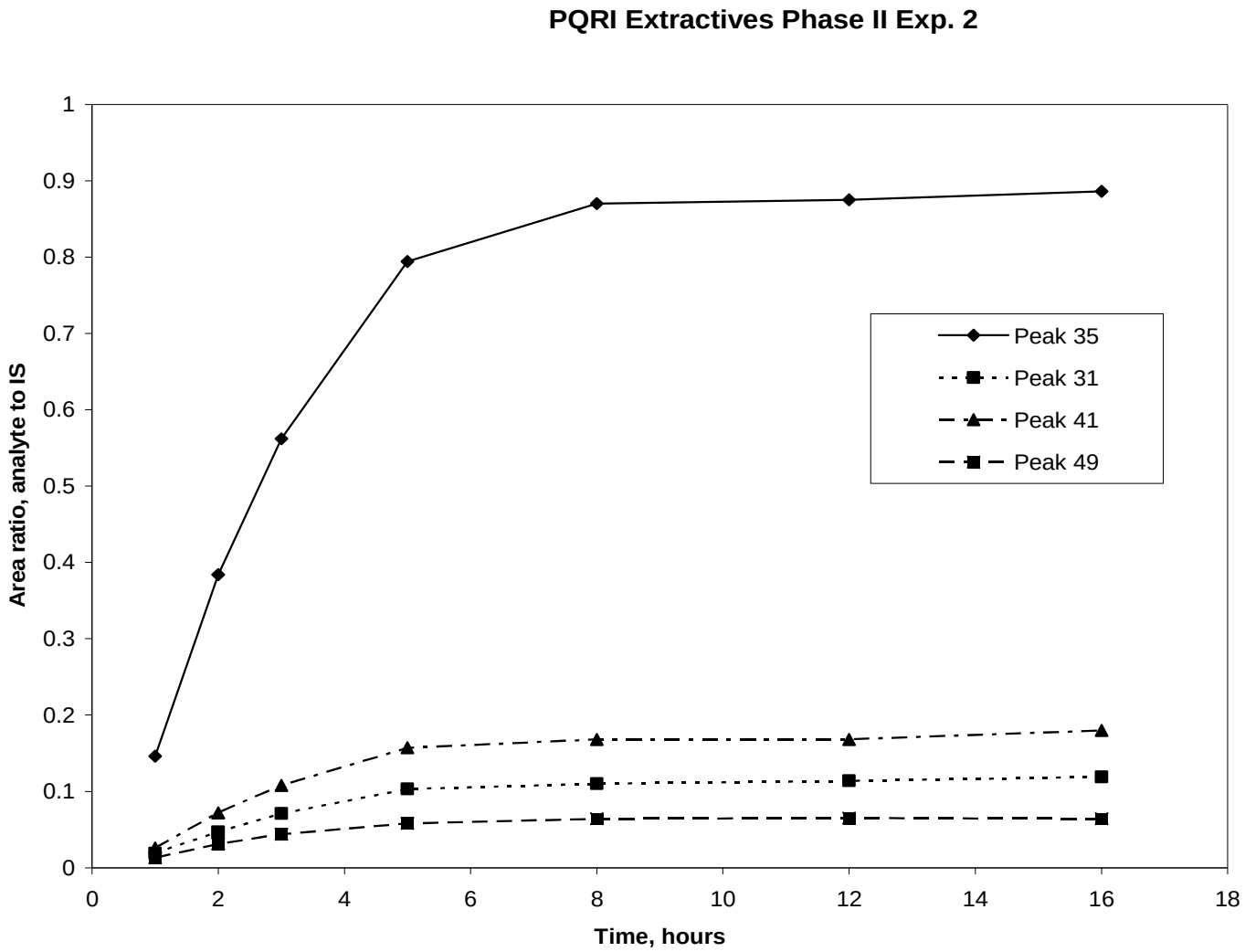


Figure 3. GC/FID extractables profile of the West sulfur-cured elastomer (test run from a preliminary GC/FID feasibility study; internal standard added).



5890 Figure 4. Model extraction optimization experiment (methylene chloride Soxhlet extraction; GC/MS analysis of extracts; internal
5891 standard added to extracting solution).



5892

Table 1. Identifications of Major Extractables from the West Sulfur-cured Elastomer (Note: peak numbers are taken from the Controlled Extraction Study results in which a total of 66 major and minor extractables were identified.)

Peak Number	Retention Time (min)	Identification	Comments
30	19.28	n-docosane	Confirmed
31	20.12	tricosane	Confirmed
33	20.94	tetracosane	Confirmed
35	21.47	2,2´-methylene-bis(6-*tert*-butyl-4-ethyl-phenol)	Confirmed (antioxidant)
36	21.73	pentacosane	Confirmed
41	22.48	hexacosane	Confirmed
45	23.20	heptacosane	Confirmed
49	23.68	Trimer (two indenes with one α-methylstyrene)	Tentative (derived from the coumarone-indene resin)
51	23.88	octacosane	Confirmed
53	24.06	Trimer (two indenes with one α-methylstyrene, containing one double-bond)	Tentative (derived from the coumarone-indene resin)
55	24.54	nonacosane	Confirmed

Note: Confirmed implies a positive match with an authentic reference material, library mass spectrum, or both.

Tentative implies a certain level of uncertainty in the exact molecular structure, however the compound class is confirmed.

Method for Quantitative Extractables Profiling of a Sulfur-Cured Elastomer Using Soxhlet Extraction and Gas Chromatography/Flame Ionization Detection

V. PURPOSE

The purpose of the method is to produce a quantitative extractables "profile" from a sulfur-cured elastomeric test article prepared for the PQRI Leachables and Extractables Working Group by West Pharmaceutical Services. The method employs a weighed sample of the elastomer test article, Soxhlet extraction of the test article with methylene chloride, an internal standard for quantitation of individual extractables via single point response factors, and analysis of the resulting methylene chloride extract by Gas Chromatography (GC) with Flame Ionization Detection (GC/FID). The resulting chromatogram is considered to be an "extractables profile".

VI. APPARATUS

250 mL round bottom boiling flasks, with ST 24/40 ground glass female joints
Soxhlet extractors, to hold a 22 x 39 mm cellulose thimble, with a male ST 24/40 joint on the bottom and a female ST 45/50 joint on top
Allihn condenser, male ST 45/50 joint on bottom
Heating mantle, to accommodate 250 mL round bottom flask
Variac or equivalent variable transformer
200 mL volumetric flasks
100 mL volumetric flasks
10 mL volumetric flasks for dilutions
250 mL graduated cylinders
Volumetric pipets (1, 2, 5, 10, 15, 20 mL, etc. as needed)

VII. REAGENTS AND STANDARDS

EM Scientific HPLC Grade methylene chloride or equivalent
2-fluorobiphenyl as the internal standard (Aldrich, 99%)
2, 2´-methylene-bis(6-*tert*- butyl-4-ethyl phenol) (Chem Services)
n-Docosane (Chem Services, 99.4%)
n-Tricosane (Chem Services, 99.2%)
n-Tetracosane (Chem Services, 99%)
n-Pentacosane (Chem Services, 99.0%)
n-Hexacosane (Chem Services, 99.2%)
n-Octacosane (Chem Services, 99.5%)
Ultra-high purity helium
Ultra-high purity hydrogen
Zero air

VIII. PREPARATION OF STANDARDS AND CALIBRATION SOLUTIONS

A. Internal Standard Spiked Extraction Solution/Calibration Diluent

This methylene chloride solution spiked with internal standard (2-fluorobiphenyl) is used to extract the elastomer samples. It is also used as a diluent for the preparation of analyte calibration standards. This extraction solution/calibration diluent preparation may be scaled up as needed. The concentration of the internal standard in this preparation is nominally100 μg/mL. This example is for a 500 mL preparation:

1. Accurately weigh approximately 50 mg of 2-fluorobiphenyl into a 500 mL volumetric flask.

2. Partially fill the flask with methylene chloride. Shake to dissolve.

3. Dilute to the mark with methylene chloride. Store at room temperature.

B. Analyte Calibration Solution (for determination of Relative Response Factors)

1. Accurately weigh approximately 10 mg of each target analyte into a 100 mL volumetric flask.

2. Add about 40 mL of calibration diluent (containing internal standard) to the volumetric and agitate to dissolve the target analytes. Note that sonication may be required to completely dissolve some of the alkanes.

3. Dilute to the mark with calibration diluent (nominal concentration 100 μg/mL for each analyte and the internal standard).

4. Pipet 1.0 mL of solution in step 3 into a 10 mL volumetric flask. Dilute with pure methylene chloride.

5. Transfer approximately 2 mL to a GC vial for analysis.

C. Linearity Solutions (for System Suitability)

Note that the actual levels and preparation procedure used for validation will be determined during method development. The following is an example.

1. Prepare a stock solution of 2, 2´-methylene-bis(6-*tert*- butyl-4-ethyl phenol) and n-pentacosane by accurately weighing 10 mg of each analyte into a 100 mL volumetric flask and bringing to volume with methylene chloride. Sonicate as required to dissolve the solid material.

2. Into individual 100 mL volumetric flasks, pipet 1.0, 2.0, 5.0, 10.0, 15.0 and 20 mL of the analyte stock solution. The levels of each analyte will be approximately 1, 2, 5, 10, 15 and 20 μg/mL.

3. Into each volumetric, pipet 10.0 mL of Internal Standard Calibration Diluent.

4. Dilute each solution to the mark with methylene chloride. The nominal concentration of internal standard is 10 μg/mL.

IX. SAMPLE PREPARATION

A. Pre-extraction of Cellulose Thimbles

1. Place about 10 boiling chips into a 250 mL round bottom flask and add approximately 200 mL of methylene chloride.

2. Place an empty cellulose thimble into a Soxhlet extractor.

3. Assemble the heating mantle, round bottom, Soxhlet, and condenser, and hook up to a Variac. Cap the unused neck of the round bottom with a ST 24/40 ground glass stopper.

4. Turn on water; observe that the water is flowing, there are no leaks and the condenser is cold.

5. Turn on Variac, to a setting between 40 and 50.

6. Pre-extract for two hours once boiling starts.

7. Allow extractor(s) to cool.

8. Properly discard the solvent.

B. Preparation and Extraction of Elastomer Sample

1. Remove the protective material from a sheet of elastomer sample (*Note: These elastomer samples were shipped in sheets from West Pharmaceutical Services wrapped in a protective material which must be removed prior to extraction.*)

2. Accurately weigh 7 ± 0.2 g of rubber sample.

3. Cut the rubber into approximately 15-25 roughly square (approximately 5 mm) pieces to fit into the bottom of the thimble. The rubber swells considerably in methylene chloride; this is to prevent the swollen rubber from protruding above the siphon in the Soxhlet, preventing full extraction.

4. Load the pieces into the pre-extracted thimble. Put the thimble into the Soxhlet.

5. Place about 10 boiling chips into a 250 mL round bottom flask.

6. Using a graduated cylinder, measure 200 mL of internal standard spiked methylene chloride into the flask.

7. Assemble the extraction apparatus as above. Turn on the water, and verify flow and that there are no leaks.

8. Turn the Variac to a setting of between 40 and 50.

9. Once boiling starts, observe the time it takes for the thimble to fill and siphon. This is the turnover time. Adjust the Variac power so that this time is between 18 and 22 min.

10. Once boiling starts, observe and record the clock time.

11. Extract under these conditions for 16 hours (Note: Extraction may be accomplished in two-eight hour increments; i.e., the extraction may be stopped after 8 hours, the system allowed to cool to room temperature, and the extraction continued for a further 8 hours the next day.)

C. Extraction Blank

Prepare an extraction blank in the same manner as the elastomer sample extract, but without the elastomer sample.

D. Sample/Blank Collection

1. After the 16 hour extraction time, turn off the Variac at the power switch without disturbing the power level dial.

2. Allow the solvent to stop boiling. This will take about 10 minutes.

3. Siphon the solvent from the Soxhlet, and clip the thimble to the top of the extractor and allow to drain.

4. Siphon last remaining solvent into the boiling flask.

5. Quantitatively transfer solution into a 200 mL volumetric flask. Rinse the boiling flask with small amounts of pure methylene chloride (no internal standard) and add these to the volumetric. Fill to the mark with methylene chloride.

6. Pipet 1.0 mL of solution in step 5 into a 10 mL volumetric flask. Dilute with pure methylene chloride.

7. Transfer approximately 2 mL to a GC vial for analysis.

X. GC CONDITIONS

Instrument: Hewlett-Packard 5890 Series II Plus, Agilent 6890, or equivalent
Column: Restek RTX-1, 30 m x 0.25 mm (0.1μm film), or equivalent
Injection Mode: Splitless

Injection Volume: 1 µL
Injector Temperature/Program: 280ºC for splitless injection
Purge Valve: On at 1.00 min; off initially
Oven Temperature: 40ºC for 1 min
40-300ºC at 10ºC/min
300ºC for 10 min
Pressure: Constant helium flow at 1.0 mL/min
Transfer line: 280ºC

XI. INJECTION SEQUENCE

1. Six (6) injections of the diluted Analyte Calibration Solution (used for determining chromatographic resolution, chromatographic tailing factor, and relative response factor precision).

2. Two (2) Injections of the Extraction Blank

3. Two (2) injections of each Linearity Solution (from low to high concentration; used for determining linearity and sensitivity).

4. Two (2) injections of each sample extract.

XII. SYSTEM SUITABILITY

A. Linearity

Evaluate linearity by plotting area ratio for each analyte in each Linearity Solution versus individual analyte concentration.

Acceptance Criteria: ***to be determined in method development***

B. Sensitivity

For each analyte in the second injection of the lowest concentration linearity solution determine signal-to-noise ratio (the term noise is taken to mean Root Mean Square noise).

Acceptance Criteria: ***to be determined in method development***

C. Chromatographic Resolution

For the second injection of the Analyte Calibration Solution, calculate the chromatographic resolution between 2, 2´-methylene-bis(6-*tert*- butyl-4-ethyl phenol) and n-pentacosane.

Acceptance Criteria: ***to be determined in method development***

D. Chromatographic Tailing Factor

For the second injection of the Analyte Calibration Solution, calculate the chromatographic tailing factors for 2, 2´-methylene-bis(6-*tert*- butyl-4-ethyl phenol) and n-pentacosane.

Acceptance Criteria: ***to be determined in method development***

E. Relative Response Factor Precision

Calculate relative response factors (RRFs) for all individual analytes for each injection of the Analyte Calibration Solution and then determine means and relative standard deviations for RRFs for each individual analyte.

$RRF = A_a \times C_i / A_i \times C_a$

where:

A_a = Peak area for an individual analyte
A_i = Peak area for the internal standard
C_a = Concentration of an individual analyte
C_i = Concentration of the internal standard

Acceptance Criteria: ***to be determined in method development***

XIII. CALCULATION OF ANALYTE LEVELS IN THE ELASTOMER SAMPLE

For each individual analyte, use the mean RRF determined in the System Suitability section (VIII.E.,).

1. Calculate the concentration of each individual analyte in the extraction solution as follows:

$C_a = A_a \times C_i / A_i \times RRF$

2. Calculate the total mass of each individual analyte in the solution as follows:

Total mass = conc.of analyte in µg/mL x 200 mL

3. Calculate the amount of each individual analyte in the elastomer as follows:

Analyte (µg/g elastomer) = Total mass of an analyte(µg)/Mass of elastomer (g)

8 September 2006

PROTOCOL ADDITIONS

PHASE 2 STUDIES: QUANTITATIVE EXTRACTABLES STUDIES ON SULFUR-CURED ELASTOMER AND POLYPROPYLENE

TABLE OF CONTENTS

Validation of a Quantitative Gas Chromatography Method for Sulfur-Cured Elastomer Extractables

I. INTRODUCTION AND BACKGROUND

Qualitative Controlled Extraction studies guided by a specific and detailed protocol have been accomplished on a sulfur-cured elastomeric test article of known additive composition. These qualitative studies produced extractables profiles by GC/MS (Gas Chromatography/Mass Spectrometry) and LC/MS (High Performance Liquid Chromatography/Mass Spectrometry) which exactly reflect the known additive composition of the elastomeric test article.

This protocol addition is designed to extend the qualitative controlled extraction study to a quantitative controlled extraction study, with appropriate method optimization and investigation of validation parameters.

II. METHOD DEVELOPMENT

Based on the results of the qualitative controlled extraction studies, Soxhlet extraction in methylene chloride with quantitative GC analysis of extracts has been selected for optimization and validation. Internal standardization utilizing appropriate authentic reference materials will be employed for quantitative calibration of the analytical system. The known additives in the elastomeric test article which can be quantitated by this analytical technique include:

2, 2’ -methylene-bis (6-*tert*- butyl-4-ethyl phenol)
Coumarone-Indene Resin related species
n-alkanes derived from paraffin
additional relatively minor extractables

All details of the analytical method, including the extraction procedure and analysis system will be documented in laboratory notebooks and/or other appropriate documentation media.

Prior to method validation, the extraction procedure will be optimized to produce maximum quantities of target extractables (i.e., “asymptotic” levels). The optimized extraction conditions will be documented and taken to method validation.

III. VALIDATION PARAMETERS AND ACCEPTANCE CRITERIA

The following validation parameters which include appropriate acceptance criteria will be investigated. When appropriate, the following model extractables will be employed:

2, 2’ -methylene-bis (6-*tert*- butyl-4-ethyl phenol)
Docosane
Hexacosane
Nonacosane
Internal Standard: 2-fluorobiphenyl

A. System Suitability

1. Instrument Precision

A test solution of target extractables with internal standard will be prepared at concentrations demonstrated not to produce adverse effects on chromatographic performance. Utilizing optimized chromatography conditions, six (6) replicate injections of the test solution will be analyzed. Peak area and area ratio measurements of target extractables and the internal standard will be determined, and means and percent relative standard deviations (%RSDs) of area ratios and relative response factors will be calculated.

Acceptance Criteria: %RSDs for area ratios ≤ 10%

2. Chromatographic Resolution

Utilizing the analyses accomplished for Instrument Precision, chromatographic resolution between appropriate peak pairs will be determined. Means and percent relative standard deviations (%RSDs) will be calculated.

Acceptance Criteria: to be determined

3. Chromatographic Tailing Factor

Utilizing the analyses accomplished for Instrument Precision, chromatographic tailing factors for appropriate peaks will be determined. Means and percent relative standard deviations (%RSDs) will be calculated.

Acceptance Criteria: to be determined

B. Linearity and Range

Linearity and range will be determined by analyzing target extractables at six (6) different concentration levels (in duplicate), over a range established during the qualitative phase of the controlled extraction study.

Acceptance Criteria: to be determined

C. Precision

1. Repeatability

Utilizing optimized extraction procedures, six (6) separate extractions will be accomplished and target extractables quantitated with the analytical method. Means and percent relative standard deviations (%RSDs) of individual target extractable amounts will be calculated.

Acceptance Criteria: %RSD for each target extractable ≤ 10%

2. Intermediate Precision

Intermediate Precision will be evaluated by a second analyst accomplishing the Repeatability study utilizing a different chromatographic system (including mobile phase and GC column). A different analytical instrument will also be utilized if available.

Acceptance Criteria: 1. %RSD for each target extractable ≤ 10%

2. %difference between analyst means for each target extractable ≤ 25%

D. Specificity

Specificity was demonstrated in the qualitative phase of the controlled extraction studies utilizing GC/MS (Gas Chromatography/Mass Spectrometry).

Acceptance Criteria:..... Confirms peak identifications and confirms no coeluting peaks for each target extractable.

E. Accuracy

Accuracy will be expressed as the percent recovery of known amounts of target extractables spiked into the extraction system.

Spiking solutions of appropriate target extractables will be prepared and spiked at three different levels (in triplicate). The individual spiking levels will be chosen to represent the appropriate range of analyte concentrations expected based on the method development experiments. Spiked samples will be analyzed by the optimized analytical method and individual mean recoveries determined for each spiking level.

Acceptance Criteria: Mean recovery for each target extractable at each spiking level should be between 80% and 120% of known spiking level.

F. Limit of Quantitation (LOQ)

A standard solution of target extractables designed to produce a response of approximately ten (10) times the LOQ (i.e., a response that provides a signal-to-noise (RMS) ration (S/N) of approximately 100:1) will be analyzed six (6) times by the optimized analytical method. Based on the average signal-to-noise ratios for each target extractable, LOQs will be estimated by extrapolation (S/N 10:1). Based on these extrapolated LOQs, a solution of target extractables will be prepared and analyzed six (6) times for LOQ confirmation.

Acceptance Criteria: Report results based on extrapolated LOQs

G. Robustness

Since there is no intention to transfer this analytical method to other laboratories, robustness experiments will not be accomplished as a part of this validation protocol.

8 September 2006

Quantification of Mercaptobenzothiazole Compounds from Sulfur Cured Rubber

I. PURPOSE

To quantify Mercaptobenzothiazole (MBT) and 2,2'-dibenzothiazyl di-sulfide (MBTS) from the extracts of sulfur cured rubber using both HPLC and LC-MS. Two extraction procedures will be compared for the extraction efficiency.

II. REFERENCE STANDARDS, SOLVENTS AND SAMPLES

Mercaptobenzothiazole (MBT), Aldrich
2,2'-dibenzothiazyl di-sulfide (MBTS), Aldrich
Methyl tert-butyl ether (MTBE)
Methylene Chloride
Sulfur cured rubber

III. INSTRUMENTATION

· Soxhlet Extraction apparatus

· Ultrasonication Bath

· Agilent 1100 series HPLC system equipped with Ultra-Violet Detector

· PE Sciex API-2000 Triple-Quadrapole Mass Spectrometry equipped with APCI source.

IV. EXTRACTION PROCEDURE

(Note: extraction conditions can be modified to obtained better recovery)

A. Sonication

Approximately 1 gram of rubber sample, cut into small pieces, and 10 ml of Methyl tert-butyl ether (MTBE) will be transferred into a suitable glass vial with screw caps. The vial will be sonicated for 30 minutes in an ultrasonication bath. Triplicate sample extraction will be performed.

B. Soxhet Extraction

Approximately 2 gram of rubber sample, cut into small pieces, will be transferred into a cellulose thimble and extracted with methylene chloride in a Soxhlet extraction apparatus for 24 hours. Triplicate sample extraction will be performed.

V. STANDARD AND SAMPLE PREPARATION

A. Reference Standard Solutions

Mixture of MBT and MBTS will be prepared at five concentration levels between 0.1 - 10 μg/mL in acetonitrile.

1. Sample Solution

The MTBE extract from the sonication will be evaporated to dryness under nitrogen stream and reconstituted into 1 mL of acetontrile. The methylene chloride extract from the Soxhlet extraction will be brought to 200 mL in volume and 50 mL of the extract will be evaporated to dryness and reconstituted into 1 mL acetonitrile.

VI. ANALYTICAL METHODS

1. HPLC-UV

Column: Symmetry C18, 2.1 x 50 mm, 3.5 μm
Column temperature: 40°C
Autosampler temperature: Ambient
Diluent: 60: 40 acetonitrile:water, v/v
Detection wavelength: UV@280, 325 nm
Flow Rate: 0.4 ml/min
Injection volume: 20 ml
Run time: 35 minutes
Mobile phase: A:0.02 M sodium acetate buffer, pH 3.5
B:Acetonitrile
Gradient profile:

Time	MP(A)	MP(B)
0	80	20
10	20	80
20	20	80
21	80	20
35	80	20

2. LC-MS

Column: Symmetry C18, 2.1 x 50 mm, 3.5 µm
Column temperature: 40°C
Autosampler temperature: Ambient
Diluent: 60: 40 acetonitrile:water, v/v
Flow Rate: 0.4 ml/min
Injection volume: 20 ml
Run time: 35 minutes
Mobile phase: A: 0.1% formic acid
B:Acetonitrile
Gradient profile:

Time	MP(A)	MP(B)
0	80	20
10	20	80
20	20	80
21	80	20
35	80	20

Mass Spectrometer

Ionization mode: **Positive APCI**
Detection mode: **SIM @ m/z 168**

VII. QUANTITATION

The area response of the working standard solutions will be plotted against their corresponding concentration. The concentration of the extract sample solution will be calculated against the curve and converted to micro-gram per gram of rubber (ppm) based on the extraction solvent volume and concentration factors. If the area response of the sample is out of the working curve range, the sample solution will be diluted accordingly to fit into the working curve range.

VIII. REFERENCES

Hansson et al. (1997), *Contact Dermatitis*, 36, 195-200

Gaind et al, (1993) *Journal of Analytical Toxicology*, 17, 34-37.

Validation of a Quantitative High Performance Liquid Chromatography-Ultraviolet Detection Method for Polypropylene Extractables

I. INTRODUCTION AND BACKGROUND

Qualitative Controlled Extraction studies guided by a specific and detailed protocol have been accomplished on a polypropylene test article of known additive composition. These qualitative studies produced extractables profiles by GC/MS (Gas Chromatography/Mass

Spectrometry) and HPLC/DAD (High Performance Liquid Chromatography/Diode Array Detection) which exactly reflect the known additive composition of the polypropylene test article as well as showing oligomer patterns indicative of polypropylene.

This protocol addition is designed to extend the qualitative controlled extraction study to a quantitative controlled extraction study, with appropriate method optimization and investigation of validation parameters.

II. METHOD DEVELOPMENT

Based on the results of the qualitative controlled extraction studies, reflux extraction in 2-propanol with quantitative HPLC/DAD (High Performance Liquid Chromatography/Diode Array Detection) analysis of extracts has been selected for optimization and validation. External standardization utilizing appropriate authentic reference materials will be employed for quantitative calibration of the analytical system. The known additives in the polypropylene test article which can be quantitated by this analytical technique include:

Millad 3988 1,3:2,4-bis(3,4-dimethylbenzylidene)sorbitol
Ultranox 626 Bis(2,4-di-*tert*-butylphenyl)pentaerythritol diphosphite
Irganox 1010 Tetrakis(methylene-3-(3',5'-di-*tert*-butyl-4'-hydroxyphenyl)propionate) methane

All details of the analytical method, including the extraction procedure and analysis system will be documented in laboratory notebooks and/or other appropriate documentation media.

Prior to method validation, the extraction procedure will be optimized to produce maximum quantities of target extractables (i.e., "asymptotic" levels). The optimized extraction conditions will be documented and taken to method validation.

III. VALIDATION PARAMETERS AND ACCEPTANCE CRITERIA

The following validation parameters which include appropriate acceptance criteria will be investigated.

A. System Suitability

1. Instrument Precision

A test solution of target extractables will be prepared at concentrations demonstrated not to produce adverse effects on chromatographic performance. Utilizing optimized chromatography conditions, six (6) replicate injections of the test solution will be analyzed. Peak area measurements of target extractables will be determined, and means and percent relative standard deviations (%RSDs) of area ratios and relative response factors will be calculated.

Acceptance Criteria: %RSD NMT 5

2. Chromatographic Resolution

Utilizing the analyses accomplished for Instrument Precision, chromatographic resolution between appropriate peak pairs will be determined. Means and percent relative standard deviations (%RSDs) will be calculated.

Acceptance Criteria: Halfwidth Resolution NLT 2

3. Chromatographic Tailing Factor

Utilizing the analyses accomplished for Instrument Precision, chromatographic tailing factors for appropriate peaks will be determined. Means and percent relative standard deviations (%RSDs) will be calculated.

Acceptance Criteria: Tailing Factor NMT 2

B. Linearity and Range

Linearity and range will be determined by analyzing target extractables at six (6) different concentration levels (in duplicate), over a range established during the qualitative phase of the controlled extraction study.

Acceptance Criteria: Correlation Coef. 0.99

C. Precision

1. Repeatability

Utilizing optimized extraction procedures, six (6) separate extractions will be accomplished and target extractables quantitated with the analytical method. Means and percent relative standard deviations (%RSDs) of individual target extractable amounts will be calculated.

Acceptance Criteria: %RSD NMT 15

2. Intermediate Precision

Intermediate Precision will be evaluated by a second analyst accomplishing the Repeatability study utilizing a different chromatographic system (including mobile phase and HPLC column). A different analytical instrument will also be utilized if available.

Acceptance Criteria: %RSD NMT 15 and % Absolute Difference of the mean between Analyst *1 and 2 is NMT 15*

D. Specificity

Specificity was demonstrated in the qualitative phase of the controlled extraction studies utilizing HPLC/DAD and LC/MS (Liquid Chromatography/Mass Spectrometry).

Acceptance Criteria: Confirms peak identifications and confirms no coeluting peaks for each target extra extractable

E. Accuracy

Accuracy will be expressed as the percent recovery of known amounts of target extractables spiked into the extraction system.

Spiking solutions of appropriate target extractables will be prepared and spiked at three different levels (in triplicate). The individual spiking levels will be chosen to represent the appropriate range of analyte concentrations expected based on the method development experiments. Spiked samples will be analyzed by the optimized analytical method and individual mean recoveries determined for each spiking level.

Acceptance Criteria: Mean recovery for each target extractable at each spiking level should be between 80% and 120% of known spiking level.

F. Limit of Quantitation (LOQ)

A standard solution of target extractables designed to produce a response of approximately ten (10) times the LOQ (i.e., a response that provides a signal-to-noise (RMS) ration (S/N) of approximately 100:1) will be analyzed six (6) times by the optimized analytical method. Based on the average signal-to-noise ratios for each target extractable, LOQs will be estimated by extrapolation (S/N 10:1). Based on these extrapolated LOQs, a solution of target extractables will be prepared and analyzed six (6) times for LOQ confirmation.

Acceptance Criteria: Report results based on extrapolated LOQs

G. Robustness

Since there is no intention to transfer this analytical method to other laboratories, robustness experiments will not be accomplished as a part of this validation protocol.

Appendix to Protocol Addition

Draft Method For Extractables Profiling of a Sulfur-Cured Elastomer Using Soxhlet Extraction And Gas Chromatographic Analysis

IV. INTRODUCTION AND BACKGROUND

This extractables profiling method was developed in support of investigational studies undertaken by the PQRI Leachables and Extractables Working Group (Product Quality Research Institute). The purpose of the method is to produce a quantitative extractables "profile" from a sulfur-cured elastomeric test article prepared for the Working Group by West Pharmaceutical Services. The method employs Soxhlet extraction with methylene chloride of a weighed sample of the elastomer test article, followed by analysis of the resulting extract by Gas Chromatography (GC). The resulting chromatogram is considered to be an "extractables profile". An internal standard (2-fluorobiphenyl) is used for quantitation of individual extractables.

V. APPARATUS AND EQUIPMENT

Analytical balance, capable of weighing to 0.00001g
Wax-coated weighing paper.

For each extraction:

250 mL round bottom boiling flasks, with two ST 24/40 ground glass female joints
Soxhlet extractors, to hold a 22 x 39 mm cellulose thimble, with a male ST 24/40 joint on the bottom and a female ST 45/50 joint on top
Allhin condenser, male ST 45/50 joint on bottom
ST 24/40 ground glass stoppers
Teflon or glass boiling chips
Cold tap or recirculated water
Tygon tubing to connect condensers to tap and together
Heating mantle, to accommodate 250 mL round bottom flask
Variac or equivalent variable transformer
Cellulose thimbles, 33 x 80 mm, Schleicher 7 Schuell or equivalent
Glass volumetric pipets, 0.5 mL
Pipet bulbs or automatic pipettor
Glass volumetric flasks with ground glass stoppers (5 mL)
250 mL glass graduated cylinder
Ring stands, monkey bars, or equivalent to hold extractors
Clamps and clamp holders
Disposable 5 ¾" glass pipets
2 mL rubber bulbs

For GC/MS or GC/FID:

Hewlett-Packard 5890 Series II Plus, Agilent 6890, or equivalent gas chromatograph, equipped with an MSD and/or an FID

Restek RTX-1 30 m x 0.25 mm (0.1μm film) GC column or equivalent
2 mL glass vials, caps and fluoropolymer lined septa

VI. CHEMICALS/REAGENTS

EM Scientific HPLC Grade methylene chloride or equivalent
2-fluorobiphenyl (Aldrich, 99%)
Ultra-high purity helium
Ultra-high purity hydrogen
Zero air

VII. PREPARATION OF INTERNAL STANDARD SPIKED EXTRACTION SOLUTION

This may be scaled up as needed. The concentration of the internal standard is approximately 100 μg/mL. This example is for 500 mL of internal standard solution.

1. Accurately weigh approximately 50 mg of 2-fluorobiphenyl into a 500 mL volumetric flask.
2. Partially fill the flask with methylene chloride. Shake to dissolve.
3. Dilute to the mark with methylene chloride. Store at room temperature.

VIII. PRE-EXTRACTION OF CELLULOSE THIMBLES

1. Place about 10 boiling chips into a 250 mL round bottom flask and add approximately 200 mL of methylene chloride.
2. Place an empty cellulose thimble into a Soxhlet extractor.
3. Assemble the heating mantle, round bottom, Soxhlet, and condenser, and hook up to a Variac. Cap the unused neck of the round bottom with a ST 24/40 ground glass stopper.
4. Turn on water; observe that the water is flowing, there are no leaks and the condenser is cold.
5. Turn on Variac, to a setting between 40 and 50.
6. Pre-extract for two hours once boiling starts.
7. Allow extractor(s) to cool.
8. Properly discard the solvent.

B. Preparation and Extraction of Rubber Sample

1. Remove any release liner/coating from the rubber.

2. Tare a piece of wax weighing paper.

3. Cut the rubber so that it fits on the weighing paper. Add or remove portions to get to 7 ±0.2 g; weigh to nearest 0.00001 g.

4. Cut the rubber into approximately 15-25 roughly square pieces to fit into the bottom of the thimble. The rubber swells considerably in methylene chloride; this is to prevent the swollen rubber from protruding above the siphon in the Soxhlet, preventing full extraction.

5. Load the pieces into the pre-extracted thimble. Put the thimble into the Soxhlet.

6. Place about 10 boiling chips into a 250 mL 2-neck round bottom flask.

7. Using a graduated cylinder, measure 200 mL of internal standard spiked methylene chloride into the flask.

8. Assemble the extraction apparatus as above. Cap the unused port with a ST 24/40 ground glass stopper.

9. Turn on the water, and verify flow and that there are no leaks.

10. Turn the Variac to a setting of between 40 and 50.

11. Once boiling starts, observe the time it takes for the thimble to fill and siphon. This is the turnover time. Adjust the Variac power so that this time is between 18 and 22 min.

12. Once boiling starts, observe and record the clock time.

13. Extract under these conditions for 16 hours (Note: Extraction may be accomplished in two-eight hour increments; i.e., the extraction may be stopped after 8 hours, the system allowed to cool to room temperature, and the extraction continued for a further 8 hours the next day.)

IX. SAMPLE COLLECTION

1. After the 16 hour extraction time, turn off the Variac at the power switch without disturbing the power level dial. Record the clock time.

2. Allow the bulk of the fluid to stop boiling. This will take about 10 minutes.

3. Remove the ground glass stopper.

4. Using a glass 0.5 mL glass volumetric pipet, remove 0.5 mL of extract and transfer it to a 5 mL volumetric flask.

5. Dilute the extract to the mark with pure methylene chloride. *Do not use the internal standard solution.* Shake to mix.

6. Using a glass disposable pipet, transfer a portion of the diluted extract to a 2 mL glass vial. Cap the vial with a fluoropolymer-lined septum and cap.

7. Collect GC/MS or GC/FID chromatogram.

X. GAS CHROMATOGRAPHY WITH MSD OR FID

GC conditions are:

Instrument: Hewlett-Packard 5890 Series II Plus, Agilent 6890, or equivalent
Column: Restek RTX-1, 30 m x 0.25 mm (0.1μm film), or equivalent
Injection Mode: Splitless
Injection Volume: 1 μL
Injector Temperature/Program: 280ºC for splitless injection
Purge Valve: On at 1.00 min; off initially
Oven Temperature: 40ºC for 1 min
40-300ºC at 10ºC/min
300ºC for 10 min
Pressure: Constant helium flow at 1.0 mL/min
Transfer line: 280ºC

If a mass spectrometer is used:

Instrument: HP 5972, Agilent 5973 MSD or equivalent
Ionization Mode: EI (Electron Ionization)
Scan Mode: Scanning; m/z 50-650
Scan Cycle Time: ..... Approx. 2 seconds/scan

XI. CALCULATIONS (FOR DATA COLLECTED BY MASS SPECTROMETRY)

1. Using the selected ion extraction menu, select ions of M/Z 172 (2-fluorobiphenyl); 191 (phenolic); 71 (hydrocarbons) and 233 (coumarone-indene.)

2. Integrate each selected ion chromatograph.

3. Calculate the ratio between each analyte peak area and that of the internal standard. For the hydrocarbons, it is useful to select one well resolved peak to either side of the phenolic peak. In this work, docosane (C22) and hexacosane (C26) are used.

4. Plot the ratio vs time for each analyte.

5. Select an extraction time well onto the asymptotic part of the curve.

APPENDIX 5

WORK PLAN

Leachables and Extractables Working Group

Spring 2002

PROPOSED WORK PLAN

Development of Scientifically Justifiable Thresholds for Leachables and Extractables

Finalized by Working Group on 20 February 2002

Forwarded for review to DPTC on 20 February 2002
Approved by DPTC on 25 April 2002

Forwarded for review to SC on 25 April 2002
Approved by SC on 26 April 2002

TABLE OF CONTENTS

I. BACKGROUND

Leachables in orally inhaled and nasal drug products (OINDP) are compounds which are present in the drug product due to leaching from container closure system components. Extractables are compounds that can be extracted from OINDP device components, or surfaces of the OINDP container closure system when in the presence of an appropriate solvent(s) and/or condition(s). Leachables are often a subset of, or are derived directly or indirectly from extractables. Extractables may, therefore, be considered as potential leachables in OINDPs. Some leachables may affect product quality and/or present potential safety risks, therefore regulatory guidance has provided some recommendations regarding the analysis and toxicological safety assessment (i.e., qualification) of such compounds.

In November 1998 and May 1999, the FDA issued two CMC draft Guidances addressing OINDP: (i) the draft Metered *Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products Chemistry, Manufacturing, and Controls Documentation*[1] (referred to here as the "MDI/DPI draft Guidance"); and (ii) the draft *Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products Chemistry, Manufacturing, and Controls Documentation*[2] (referred to here as the "Nasal Spray draft Guidance").

Currently, the draft Guidances recommend that the sponsor identify, report, and conduct toxicological analyses on all extractables found in the controlled extraction study (referred to in the draft Guidances as a "control extraction study"). Examples of these recommendations are described in the draft MDI/DPI Guidance regarding MDI canisters, valves, and actuators (lines 883-884; 990-991; and 1073):

> *…the profile of each extract should be evaluated both analytically and toxicologically.*

This recommendation is problematic because it suggests that *all extractables* must be reported and undergo toxicological safety assessments. However, some of these extractables may not be present in the final drug product (i.e., they are not leachables), or may exist as leachables at levels so low as to be of negligible risk to human safety. Thus, the draft guidances appear to recommend toxicological assessments on compounds for which the patient will either never be exposed, or which might exist at levels that present negligible safety risk. Further, the draft Guidances do not offer advice as to the concentration levels (i.e., thresholds) at which extractables/leachables should be identified, quantified, reported, and qualified for safety purposes.

II. RESEARCH OBJECTIVE

A. Why Work is Being Done

Regulatory and industry resources will have greatest impact when focussed on toxicological issues related to those compounds that are introduced to the patient (i.e., leachables), as well as consideration of the levels of such compounds that may affect human safety. A logical way to address this is to develop thresholds for reporting and safety qualification of *leachables*.

A reporting threshold with associated identification and quantitation thresholds for leachables would be established to support toxicological safety qualification. A qualification threshold

would establish a limit below which the leachable is not considered for safety qualification unless it presents structure-activity relationship (SAR) concerns. Note that certain classes of potential leachable compounds with special toxicological concerns [e.g., nitrosamines, polynuclear aromatics (PNAs), mercaptobenzthiazole, etc.] would require development of reporting thresholds on a case-by-case basis. Both these thresholds assume that toxicological qualification should be performed on leachables and not on extractables.

The establishment of reporting and qualification thresholds for leachables would then naturally lead to reporting thresholds for extractables. This would facilitate the development of appropriate quality control strategies for extractables at the component level, which would then in turn provide indirect control of leachables in drug products without the need for routine analytical testing of leachables.

B. Hypothesis

Based on the above discussion, the following working hypothesis is proposed:

1. *Scientifically justifiable thresholds based on the best available data and industry practices can be developed for:*

   (a) *the reporting and safety qualification of leachables in orally inhaled and nasal drug products, and*

   (b) *reporting of extractables from the critical components used in corresponding container/closure systems.*

   Reporting thresholds for leachables and extractables will include associated identification and quantitation thresholds.

2. *Safety qualification of extractables, would be scientifically justified on a case-by-case basis.*

The work plan outline described below is designed to test this hypothesis through a process intended to develop these scientifically justifiable thresholds.

C. Work Plan Outline

The essence of the proposed Work Plan is that in order to test the hypothesis that appropriate and scientifically justifiable thresholds exist, then the Working Group must engage in a process designed to develop these thresholds. It is envisioned that processes designed to develop qualification and reporting thresholds would proceed somewhat in parallel, with the former taking advantage of the toxicological expertise of particular Working Group members and the latter taking advantage of the analytical chemistry expertise of others in the Group. It is also considered likely that the development of reporting thresholds will require example data in the form of leachables and extractables profiles, etc., from various OINDPs. These data will be utilized to explore important concepts such as “correlation” of leachables and extractables. Every effort will be made to solicit appropriate existing data (industry, academic, or government

sources), and as required to generate new data in laboratory facilities available to Working Group members, or others within PQRI.

The following Work Plan is proposed to test the hypothesis stated above:

Task 1: Process Development

Goal: The Working Group will agree on the outline of a process (or processes) designed to test the stated hypothesis by attempting to develop appropriate and scientifically justifiable qualification and reporting thresholds related to leachables and extractables.

Implementation: The ITFG/IPAC-RS Collaboration engaged in a process which resulted in qualification thresholds for leachables, and reporting thresholds for extractables and leachables. These proposed thresholds and the processes used to develop them are described in the document *Points to Consider*.[3]

In its second face-to-face meeting, the Working Group will review the processes described in *Points to Consider* and through its own deliberation, design and agree on the outlines of processes that it will employ for threshold development. ITFG/IPAC-RS representatives who are also members of the Working Group will present and describe the processes that they employed for threshold development. It should be emphasized that the *Points to Consider* document will be used as a model for process development only. The Working Group will not at this point consider or debate the actual numerical thresholds proposed in this document. It is envisioned that the additional expertise and perspective available in the Working Group will result in enhanced processes for threshold development.

Outcome: The expected outcome from *Task 1* is the outline of a process(es) designed to develop qualification and reporting thresholds, and thereby test the hypothesis.

Timeline: **1 May 2002** for completion of *Task 1*.

Required Resources: It is envisioned that *Task 1* will require only facilities for face-to-face meeting(s) and teleconferences.

Task 2: Process Implementation

Threshold development can be logically divided into two separate but related sub-tasks: (1) development of qualification thresholds and (2) development of reporting thresholds. It is envisioned that these two processes will proceed in parallel utilizing appropriate expertise from various Group members, with clear and continuous communication between the two sub-tasks.

(1) Sub-task: Development of Qualification Thresholds

Goal: The Working Group will develop appropriate and scientifically justifiable qualification thresholds for leachables. A qualification process will be developed for extractables which can be employed as required on a case by case basis.

Implementation: The Working Group will employ the process outline from *Task 1* to develop qualification thresholds. The Group will consider and debate many questions during this process. Examples of these questions are as follows:

- Is it appropriate to use exposure standards for environmental pollutants for developing a qualification threshold for leachables/extractables in OINDP?
- Is there utility in other qualification threshold strategies, e.g., indirect food additive regulations) for OINDP application?
- Is there utility to be found from other sources, e.g., USP, ISO 10993, 21 CFR (174-178)) regarding risk assessment, qualification, and thresholding of leachables/extractables?
- What are the testing paradigms that could provide data for risk assessment of leachables/extractables in OINDP?
- Is there utility in the testing procedures described in USP<87> and <88> for safety qualification of any OINDP?
- Is there utility in considering other available qualification decision trees, e.g., ICH guideline for impurities) for the qualification of leachables/extractables?

The Working Group will develop a qualification strategy for leachables that will include testing strategies, risk assessment models, and decision trees; as appropriate.

Once the qualification strategy is generally agreed upon, the Working Group will devise a generic list of potential leachables for a "worst case scenario" OINDP. The compounds on the list and their exposure levels to patients will be based on the expertise and knowledge-base of Working Group members, and information solicited from represented industry/academic/government organizations. The list will then be used for a mock toxicological qualification and risk assessment to test the credibility of a qualification threshold. The list (termed Product X) will likely be designed to mimic an MDI (Metered Dose Inhaler) drug product which, of all OINDPs, is most likely to have an extensive leachables profile which correlates directly with its device components extractables profile(s). The Product X data set should also encompass special case leachables (i.e., nitrosamines and PNAs) as well as less often encountered leachables. The concentrations of leachables proposed for Product X should be within a range consistent with current manufacturing practices for OINDPs.

The mock toxicological qualification will assess whether the threshold argument adequately qualified leachables, as represented by the Product X profile/list. It should also determine if the proposed qualification/testing paradigm would adequately qualify leachables that fell outside the proposed threshold.

Outcome: The expected and potential outcomes from this sub-task are as follows:

- A qualification/testing paradigm for leachables/extractables in OINDPs.
- A decision tree for qualification of leachables/extractables in OINDPs.

- Thresholds for qualification of leachables/extractables in OINDPs.

- An example of a complete qualification for a representative leachables profile from a typical OINDP.

A consensus within the Working Group on qualification thresholds and the successful completion of the mock qualification will be considered a successful test of the hypothesis.

(2) Sub-task: Development of Reporting Thresholds

Goal: The Working Group will develop appropriate and scientifically justifiable reporting thresholds for extractables and leachables.

Implementation: The Working Group will employ the process outline from *Task 1* to develop reporting thresholds. The Group will consider and debate many questions during this process. Examples of these questions are as follows:

- What analytical technologies and strategies are typically used by the industry for identification and quantification of extractables and leachables? What are the relative strengths and weaknesses of these technologies and strategies? What thresholds for detection/quantification do these technologies imply? What are appropriate target compounds for development and validation of specific analytical methods for leachables/extractables? Is there any utility in methods and strategies contained in ICHQ2B, USP<381>, USP<661>, ISO 10993 (draft), and 21CFR (170-180)? Is it appropriate for the Working Group to propose/recommend most appropriate technologies/strategies for identification and quantification of various classes of extractables/leachables?

- What does it mean to "identify" an extractable/leachable? Is it appropriate for the Working Group to propose/recommend criteria for identification of extractables/leachables?

- How does one design and implement a "controlled extraction" study for extractables? Is it appropriate for the Working Group to propose/recommend a most appropriate strategy for controlled extraction studies? Will this strategy depend on the particular OINDP dosage form (MDI, DPI, etc.) and the nature of the material being extracted?

- What is a "critical component" in an OINDP?

- Is it appropriate to use extractables tests as secondary controls on the composition of critical components in an OINDP? Are there better approaches?

- What are appropriate routine control technologies/strategies for extractables? Is it appropriate for the Working Group to propose/recommend a most appropriate technology/strategy for routine control of extractables? Under what circumstances will leachables controls be required?

It is envisioned that investigation of these questions will require data in the form of extractables/leachables profiles, as well as a body of information on current industry practices. All available sources of appropriate data and information will be solicited through the Working Group members and the organizations they represent. If new laboratory studies are required to generate data, these will be solicited through the laboratories of the Working Group members or their contacts.

It is also envisioned that the Working Group will assemble an advisory team of OINDP component manufacturers to provide appropriate input and data to the process.

Outcome: The expected and potential outcomes from this sub-task are as follows:

- Recommended technologies/strategies for extractables/leachables studies.
- Recommended criteria for identification of extractables/leachables.
- Thresholds for the identification and reporting of extractables/leachables.
- Thresholds for the quantification of extractables/leachables.
- Recommended control technologies/strategies for extractables/leachables.

A consensus within the Working Group on reporting thresholds will be considered a successful test of the hypothesis.

Timeline: **1 May 2003** for completion of *Task 2* (including both sub-tasks).

Required Resources: It is envisioned that *Task 2* will require only facilities for face-to-face meeting(s) and teleconferences. Required information and data will be collected/generated with the resources available to members of the Working Group and their respective organizations and contacts.

Task 3: Harmonization and Consensus

Goal: The Working Group will thoroughly evaluate the results of the process implementation described under *Task 2* (including any data and other information employed) and come to consensus as to the validity of the hypothesis based on the testing criteria previously stated.

Implementation: The Working Group as a whole will critically evaluate the outcomes of *Task 2* and create a report for review within the PQRI process that will include all proposed outcomes as well as clearly stated recommendations for the Agency (FDA) to consider in the final implementation of their draft Guidances.

Other outcomes from *Task 3* may include publications and presentations at appropriate scientific meetings and forums. These additional outcomes will be discussed and agreed to at the appropriate time in the overall PQRI process.

Timeline: **1 September 2003** for completion of *Task 3.*

Required Resources: It is envisioned that *Task 3* will require only facilities for face-to-face meeting(s) and teleconferences. Additional required information and data will be collected/generated with the resources available to members of the Working Group and their respective organizations and contacts.

III. SUMMARY OF REQUIRED RESOURCES

A. Human Resources

Current members of the Working Group are:

Daniel L. Norwood (Boehringer Ingelheim), Chair
Gordon Hansen (Boehringer Ingelheim), PQRI Steering Committee
Doug Ball (Pfizer)
Tom Feinberg (Magellan Laboratories)
Jim Blanchard (Aradigm)
Fran DeGrazio (West)
Debby Miran (Miran Consulting)
Roxana Nikoui (Valois)
Roger McClellan (UNM)
David Porter (USP)
Diane Paskiet (Monarch Analytical)
Alan Schroeder (FDA)
Mark Vogel (Pharmacia)
Tim McGovern (FDA)

In addition, Guirag Poochikian (FDA) and Jeffery Blumenstein (Pfizer) serve as liaisons to the DPTC, and the IPAC-RS Secretariat provides administrative, logistical, and other support.

Members of the Working Group bring to the process a variety of expertise and experience, including analytical chemistry, inhalation toxicology, OINDP development, regulatory affairs, and device/drug product manufacturing. These resources will be supplemented, if required, by additional resources available to the represented organizations (i.e., IPAC-RS, PDA, *etc.*). A plan is currently under consideration by the Working Group to create an Advisory Group of OINDP component supplier/manufacturer representatives to assist the Group in the proposed project.

B. Laboratory Resources

As previously stated, required laboratory resources for the generation of original data will be solicited from the Working Group members and their contacts.

C. Financial Resources

No additional financial resources from PQRI are requested at this time. In-kind donations of resources may be solicited from the Working Group member organizations.

IV. POTENTIAL IMPACT

The establishment of reporting and qualification thresholds for leachables, and reporting thresholds for extractables, would enhance the utility of the draft Guidances, which would in turn facilitate drug development programs for OINDPs by reducing uncertainty, and thus making such programs more time and cost efficient. This would likely result in regulatory submissions of greater quality and consistency which would facilitate the review process. The end result to the patient will be continued improvement in product quality.

V. GLOSSARY

ICH Q2B	ICH guideline on validation of analytical procedures: methodology
ICH Q3B	ICH guideline on impurities in new drug products
ISO 10993	International Standard Organization: biological evaluation of medical devices
USP<1031>	USP general information chapter for biocompatibility
USP<87>	USP general test chapter for in vitro biological reactivity tests
USP<88>	USP general test chapter for in vivo biological reactivity tests
USP<381>	USP general test chapter for elastomeric closures for injections
USP<661>	USP general test chapter for containers
21CFR (170-180)	Code of Federal Regulations, volume 21, parts 170-180: food additives and indirect food additives

---

[1] *Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products* Chemistry, Manufacturing, and Controls Documentation, CDER/FDA, October 1998, (Docket No. 98D-0997), available at http://www.fda.gov/cder/guidance/2180.pdf.

[2] *Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products* Chemistry, Manufacturing, and Controls Documentation, CDER/FDA, May 1999, (Docket No. 99D-1454), available at http://www.fda.gov/cder/guidance/2836.pdf.

[3] ITFG/IPAC-RS Collaboration, Leachables and Extractables Testing: Points to Consider, available at http://www.ipacrs.com/leachables.html