SCHOTT Pharma Services
Thorsten Sogding, Daniel Canton, Daniel Haines, Uwe Rothhaar

How Sterilization of PRIMARY PACKAGING Influences the Results of E&L Studies

As the demands that are being placed on the quality and stability of medications continue to increase, the interactions that take place between the primary packaging container and filled drug product are becoming increasingly important

Even primary packaging that has been manufactured and stored properly can release substances into the drug formulation. For this reason, pharmaceutical manufacturers are required to conduct extensive studies on "Extractables and Leachables" (E&L). The compounds found in such studies depend not only on the ingredients of the packaging materials, such as antioxidants, plasticizers, antistatic agents, catalysts and cross-linking agents, but also reflect how the packaging components were treated. This study demonstrates the influence of the type of sterilization on the resulting extraction profiles based on the example of bromobutyl rubber stoppers. This demonstrates that more than validated analysis is essential to obtain meaningful results. The correct study design and comprehensive know-how on the entire life cycle of commercially available primary packaging—from the raw materials to processing and use—are crucial to determining the origin of found substances.

Packaging systems often consist of many different compo-

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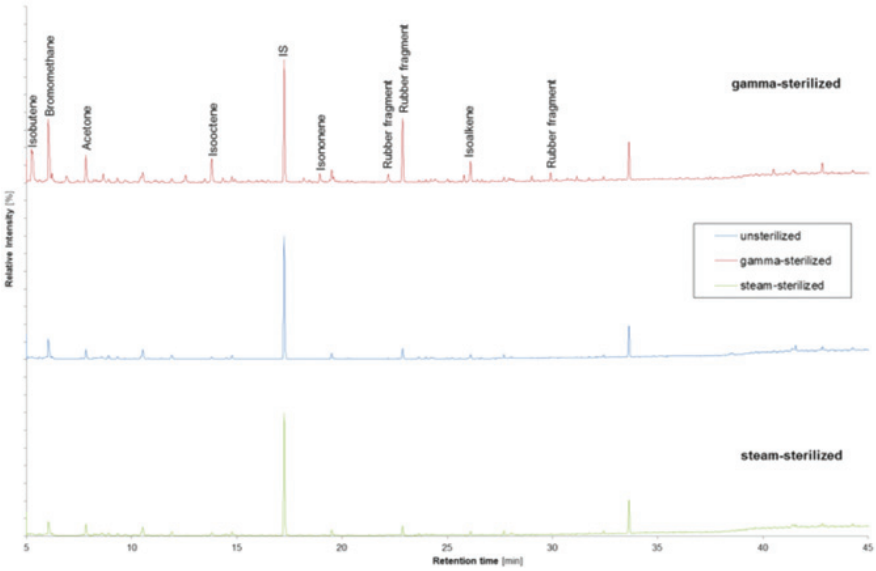
FIGURE 1: Exemplary HS-GC/MS-chromatograms (140 °C / 45 min) of unsterilized (center), steam-sterilized (below) and gamma-sterilized (above) sample D.



nents and materials, like glass, metal, plastic, rubber, adhesives or lubricants. Extractables are determined by subjecting the packaging material to aggressive conditions such as different solvents at elevated temperature for extended times, resulting in substances that elute from these packaging systems. By contrast, leachables are substances that migrate into a pharmaceutical formulation under normal preparation and storage conditions. A study on extractables represents a worst case scenario to identify as many substances as possible that could conceivably enter into the medication.

Leachables are usually, but not always, a subgroup of extractables; for instance, a substance contained in the drug product formulation can react with a constituent contained in the packaging and form an entirely new species that is later identified in a leachable study, but would not be present in an extractable study.

Pharmaceutical manufacturers are required to perform E&L studies to exclude possible harmful interactions between the packaging materials and the medication. The primary sources of organic extractables and leachables are elastomeric and polymeric materials like rubber and plastics, because they contain additives that allow for them to have beneficial properties, such as greater chemical stability and increased manufacturing yield. Here, it is important to know the exact composition of the packaging material in order to be able to perform E&L studies efficiently. Nevertheless, this type of information often cannot be obtained from the material suppliers due to the complexity of the manufacturing processes, desire to protect their own process know-how, and potential upstream changes in raw materials. To address this issue, most testing laboratories that perform E&L studies have built up comprehensive databases on materials and additives.

Most people are less aware of the fact that the original composition of the packaging system alone is not mainly responsible for what substances are found as E&L. One additional influencing factor is the sterilization process. As is well known, gamma irradiation can lead to polymer and additive degradation (e.g. crosslinking and scission), whereas steam sterilization can alter the mechanical and chemical properties as well (e.g. softening).

The following study is focused on changes in the E&L profiles of common bromobutyl stoppers caused by different sterilization procedures.

REGULATORY REQUIREMENTS

Pharmaceutical companies need to conduct leachable studies to prove that no harmful substances will penetrate from the primary packaging into the drug during appropriate use of the drug in the respective dosage under normal storage conditions. This procedure is specified in a number of guidelines or regulations for the United States/Canada[1-6] and for Europe.[7-8]

It is important for pharmaceutical manufacturers to be able to come up with this proof as cost effectively as possible. Extractable studies help them to achieve this goal because they enable the toxicological assessment of possible Leachables and identity the number of substances that need to be tested with validated methods in the subsequent leachable studies. The flow charts from recommended E&L study plans are shown in BPSA's Extractables and Leachables Subcommittee,

FIGURE 2: Concentration of isobutene, bromomethane, and acetone out of sample A after different sterilization procedures found by HS-GC/MS after incubation at 140 °C (45 min).

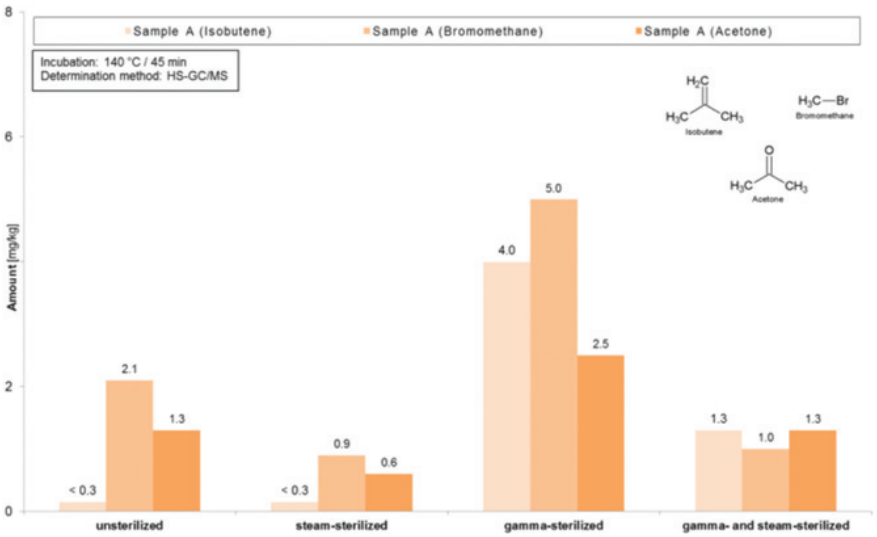


TABLE 1: Qualitative results of HS-GC/MS (VOC) analyses of samples A to E upon various sterilization processes.

Sample	Not Sterilized	Steam-Sterilized	Gamma-Sterilized	Gamma- and Steam-Sterilized
A	Significant signals: - Bromomethane - Acetone - tert-Butanol - Methylcyclopentane - C13H24 and C21H40 cyclic rubber oligomers and brominated derivates - Various hydrocarbons and unidentified compounds	Significant signals: - Bromomethane - Acetone - tert-Butanol - Methylcyclopentane - C13H24 and C21H40 cyclic rubber oligomers and brominated derivates - Various hydrocarbons and unidentified compounds	New significant signals: - Isobutene - Diisobutene - Various saturated and unsaturated hydrocarbons Increasing signals: - Acetone - Bromomethane - Various unidentified compounds (e.g. rubber fragments)	General observations: - Compared to gamma-sterilized sample, no new signals observed - Decreasing intensity of most signals, especially low boiling substances (e.g. isobutene or bromomethane) General observations: - Compared to gamma-sterilized sample, no new signals observed - Slightly decreasing intensity of some signals, especially low boiling substances (e.g. isobutene or bromomethane)
B	Significant signals: - Acetone - 2- and 3-Methylpentane - Methylcyclopentane - C13H24 and C21H40 cyclic rubber oligomers and brominated derivates - Various hydrocarbons and unidentified compounds	General observations: - No new signals observed - Slightly decreasing intensity for some single signals, especially low boiling substances (e.g. acetone or methylpentane)	New significant signals: - Isobutene - Diisobutene - Bromomethane - Various saturated and unsaturated hydrocarbons Increasing signals: - Acetone - Various hydrocarbons and some unidentified compounds (e.g. rubber fragments)	General observations: - Compared to gamma-sterilized sample, no new signals observed - Slightly decreasing intensity of some signals, especially low boiling substances (e.g. isobutene or bromomethane)
C	Significant signals: - Bromomethane - Hexane - Cyclooctane - Some unidentified compounds, probably rubber oligomers or fragments General remark: - Lowest amount of VOC compared to other samples	General observations: - No new signals observed - Decreasing intensity of most signals, especially low boiling substances (e.g. bromo-methane, hexane or cyclooctane)	New significant signals: - Isobutene - Diisobutene - Various hydrocarbons and some unidentified compounds (e.g. rubber fragments) Increasing signals: - Bromomethane - Various hydrocarbons and unidentified compounds (e.g. rubber fragments)	New significant signals: - Isobutene - Diisobutene - Various hydrocarbons and some unidentified compounds (e.g. rubber fragments) Increasing signals: - Bromomethane - Various hydrocarbons and unidentified compounds (e.g. rubber fragments)
D	Significant signals: - Bromomethane - Acetone - C13H24 and C21H40 cyclic rubber oligomers and brominated derivates - Various hydrocarbons and unidentified compounds	General observations: - No new signals observed - Slightly decreasing intensity of some signals, especially low boiling substances (e.g. bromomethane or not further specified hydrocarbons)	New significant signals: - Isobutene - Diisobutene - Various saturated and unsaturated hydrocarbons Increasing signals: - Acetone - Bromomethane - Various unidentified compounds (e.g. rubber fragments)	General observations: - Compared to gamma-sterilized sample, no new signals observed - Decreasing intensity of most signals, especially low boiling substances (e.g. isobutene or bromomethane)
E	General observations: - Compared to gamma-sterilized sample, no new signals observed - Decreasing intensity of most signals, especially low boiling substances (e.g. isobutene or bromomethane)	General observations: - No new signals observed - Decreasing intensity of most signals, especially low boiling substances (e.g. acetone or bromomethane)	New significant signals: - Isobutene - Diisobutene - Various saturated and unsaturated hydrocarbons Increasing signals: - Acetone - Bromomethane - Various unidentified compounds (e.g. rubber fragments)	General observations: - Compared to gamma-sterilized sample, no new signals observed - Decreasing intensity of most signals, especially low boiling substances (e.g. isobutene or bromomethane)

"Recommendations for Extractables and Leachables Testing, Part Two: Executing a Program," BioProcess Int. 6(1) 2008: 44-52; and "Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products: An Overview of the PQRI Recommendations," by Norwood, Paskiet and Ruberto Pharmaceutical Res. 2008; 25(4):727-739.9-10

Toxicological assessments are performed for substances found above the Analytical Evaluation Threshold (AET).[9] This AET value is expressed as the amount of given extractable per mass of component or leachables per drug product and is determined with respect to the Permitted Daily Exposure (PDE), known from toxicological exposure studies. If toxico-

logical data are not available, then the AET can be developed using the Safety Concern Threshold (SCT)[10] of 0.15 µg/day along with the dosing regimen.

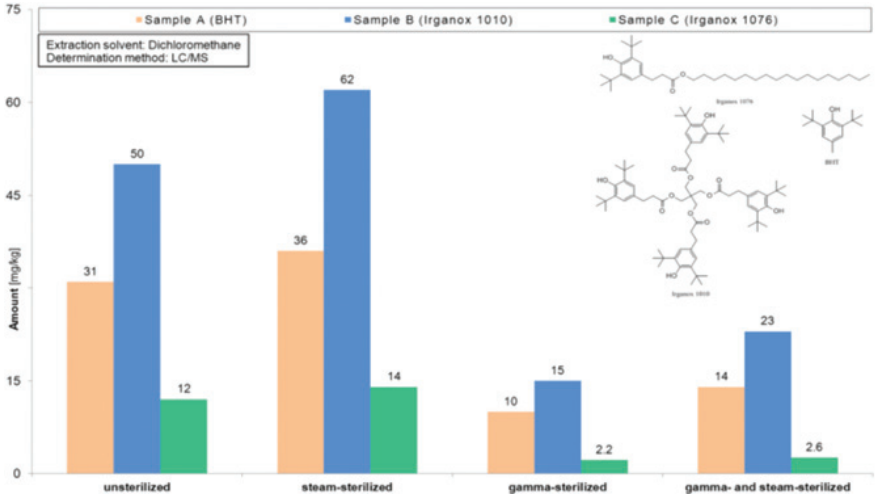
The guidelines and regulations mentioned do not include any specific instructions on how to perform E&L studies, however. A working group at the Product Quality Research Institute (PQRI) based in Arlington, VA, has developed detailed recommendations on a special group of pharmaceutical products in which the risk of interactions with the packaging is considered to be particularly high. Some examples are published with respect to the dosage form: This includes nasal sprays, oral inhalation aerosols[12] and parenteral liquids[13], as well as best practices for representative parenteral and ophthalmic packaging materials[14]. The USP is in the process of finalizing guidance chapters for extractables <USP 1663>[6] and leachables <USP 1664>.[7]

Without going into details, the following aspects of extractable studies are particularly important:

1. Extraction should take place using a plurality of solvents of different polarities and using several different types of extraction methods. Prior working knowledge of pharmaceutical formulations and packaging materials can help to minimize these efforts and choose the right solvents and methods.
2. Several different analytical techniques should be employed. Common suitable methods are: Headspace GC/MS (gas chromatography/mass spectrometry) for volatile Extractables, GC/MS for semi-volatile, LC/MS (liquid chromatography/mass spectrometry) for non-volatile or polar Extractables, and ICP-MS (inductively coupled plasma/mass spectrometry) for analyzing elemental impurities, e.g. metals from organic pigments or polymerization catalysts. With the increasing regulatory requirements, the analytical capabilities need to be improved and updated in short cycles especially with respect to lower detection and quantification limits.
3. Extensive experience, appropriate databases and reference materials, high qualified laboratory staff and proper quality control helps to interpret the data correctly to draw the proper conclusions and avoid mistakes.

After the extractable studies have been performed, substances (above AET) are selected on the basis of a toxicological assessment. For this selection suitable methods need to be developed and validated for a subsequent leachable study according to ICH15. Here, the leachable profile of the pharmaceuticals stored is detected using these validated methods in appropriate intervals—for example, 1, 3, 6, 12, 18, 24, 36 months—under ICH conditions. Furthermore, screenings are performed in parallel with the same methods to detect any possible modified or derived substance.

FIGURE 3: Concentration of DCM-extractable BHT, Irganox 1010, or Irganox® 1076 out of samples A - C after different sterilization procedures found by LC/MS.



STUDY DESIGN ON THE IMPACT OF STERILIZATION

The study was designed to investigate the impact of various sterilization techniques onto the extraction profile of commercially available stoppers made of bromobutyl rubber for use in syringes. The sterilization of the stoppers is mandatory before they can be used for medical purposes. Three variants of sterilization were chosen: Steam sterilization (autoclaving, 121 °C for 30 minutes), gamma sterilization (40 kGy), and a combination of the two techniques gamma-irradiation (first) and steam-sterilization (second).

The study included five different stoppers from three different manufacturers. A set of state of the art chromatographic tools from Shimadzu (GCMS-QP210 Ultra, LCMS-IT-TOF with ESI and APCI source) was used to conduct the analyses of the Extractables according to the following procedure:

1. Volatile organic compounds (VOC) were analyzed by headspace GC/MS after incubation of about 2 g of stopper material at 140°C for 45 min. Quantification was performed semi-quantitative against the relative response of the internal standard toluene-D8.
2. Semi-volatile (SVOC) and non-volatile (NVOC) organic components were determined after exhaustive reflux extraction of the stopper for 8 hours with three different solvents (100 cm^2 stopper surface area per 200 mL). The solvents used were isopropanol (IPA), dichloromethane (DCM) and ultrapure water. The aqueous extracts were transferred to DCM extracts by liquid-liquid extraction. The analysis of all of these extracts was conducted using GC/MS and LC/MS. Quantification was performed semi-quantitative for GC/MS (SVOC) using the response factor (RF) of the internal standard 2-fluorobiphenyl. Quantitative values for LC/MS (NVOC) were calculated using the individual substance response factor derived from reference material measurements.

This procedure was applied to stoppers without sterilization (reference) and after sterilization with the variants de-

TABLE 2: Qualitative results of GC-MS (SVOC) and LC/MS (NVOC) analyses of different extracts of samples A to E upon various sterilization processes (general statements).

Sample	Not Sterilized	Steam-Sterilized	Gamma-Sterilized	Gamma- and Steam-Sterilized
A	Significant signals: - Butylated hydroxytoluene - Palmitic and stearic acid - C13H24 and C21H40 cyclic rubber oligomers and brominated derivates - Various unidentified compounds, e.g. rubber fragment or additive degradation products	General observations: - Tendency for increasing amount of extractable SVOC Increasing signal: - Butylated hydroxytoluene (slightly) Decreasing signals: - Palmitic and stearic acid (slightly)	General observations: - No significant change in sum of extractable SVOC compared to unsterilized sample New significant signals: - Heptadecane - Various unidentified compounds, e.g. rubber fragments Decreasing signals: - Butylated hydroxytoluene (loss of more than 50 %) Increasing signals: - Palmitic and stearic acid (slightly)	General observations: - No significant new signals observed - Slight tendency for decreasing sum of SVOC observed (compared to only gamma-sterilized sample) Decreasing signals: - Palmitic and stearic acid (slightly; compared to gamma-sterilized sample) Increasing signal: - Butylated hydroxytoluene (slightly; compared to only gamma-sterilized sample)
B	Significant signals: - Irganox® 1010 - Palmitic and stearic acid - C13H24 and C21H40 cyclic rubber oligomers and brominated derivates - Various unidentified compounds, e.g. rubber fragment or additive degradation products	General observations: - Slight decrease of extractable SVOC observed - No significant new signals were found Decreasing signals: - Palmitic and stearic acid (slightly)	General observations: - Slight increase of extractable SVOC observed New significant signals: - Pentadecane - Heptadecane Decreasing signals: - Irganox® 1010 (loss of more than 60 %) Increasing signals: - Palmitic and stearic acid (slightly) - 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (aqueous extract)	General observations: - No significant new signals observed - Slight tendency for decreasing sum of SVOC observed (compared to only gamma-sterilized sample) Decreasing signals: - Palmitic and stearic acid (slightly, depending on solvent) - 7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione (aqueous extract)
C	Significant signals: - Irganox® 1076 - 2-Chloro-4-tert-pentylphenol - Palmitic and stearic acid - C13H24 and C21H40 cyclic rubber oligomers and brominated derivates - Various unidentified compounds, e.g. rubber fragment or additive degradation products	General observations: - Slight decrease of extractable SVOC observed - No significant new signals were found Decreasing signals: - Palmitic and stearic acid (slightly)	General observations: - Slight decrease of extractable SVOC observed New significant signals: - Heptadecane - Unsaturated cyclic hydrocarbons (e.g. from rubber degradation) Decreasing signals: - Irganox® 1076 (loss of more than 75 %) Increasing signals: - Palmitic and stearic acid	General observations: - No significant new signals observed - Sum of SVOC is comparable to unsterilized material
D	Significant signals: - Butylated hydroxytoluene - Palmitic and stearic acid - C13H24 and C21H40 cyclic rubber oligomers and brominated derivates - Various unidentified compounds, e.g. rubber fragment or additive degradation products	Not tested	General observations: - Slight increase of extractable SVOC observed New significant signals: - Heptadecane - Various unidentified compounds, e.g. rubber fragments Decreasing signals: - Butylated hydroxytoluene (loss of more than 50 %) Increasing signals: - Palmitic and stearic acid (slightly)	General observations: - No significant new signals observed - Slight tendency for decreasing sum of SVOC observed (compared to only gamma-sterilized sample) Decreasing signal: - Palmitic and stearic acid (slightly; compared to only gamma-sterilized sample)
E	Significant signals: - Butylated hydroxytoluene - Palmitic and stearic acid - C13H24 and C21H40 cyclic rubber oligomers and brominated derivates - Various unidentified compounds, e.g. rubber fragment or additive degradation products	General observations: - Tendency for increasing amount of extractable SVOC Increasing signal: - Butylated hydroxytoluene (slightly) Decreasing signals: - Palmitic and stearic acid (slightly)	General observations: - Slight increase of extractable SVOC observed New significant signals: - Heptadecane - Various unidentified compounds, e.g. rubber fragments Decreasing signals: - Butylated hydroxytoluene (loss of more than 50 %) Increasing signals: - Palmitic and stearic acid (slightly)	General observations: - No significant new signals observed - Slight tendency for decreasing sum of SVOC observed (compared to only gamma-sterilized sample) Decreasing signals: - Palmitic and stearic acid (slightly; compared to only gamma-sterilized sample)

scribed. The substances detected in the chromatograms and the respective fragmentation patterns were identified using a combination of commercially available databases and our internal reference database on packaging products and additives.

SUMMARY OF THE RESULTS

Because of the study design—5 stoppers, 3 different sterilization variants, 3 solvents and headspace—a large data set was generated that cannot be fully reported here; (the full study can be obtained by request from the authors). Therefore, a comprehensive summary is given for each stopper type (A – E) in Tables 1 and 2 and some exemplary chromatograms and evaluation diagrams for selected extractables are depicted in Figures 1-4.

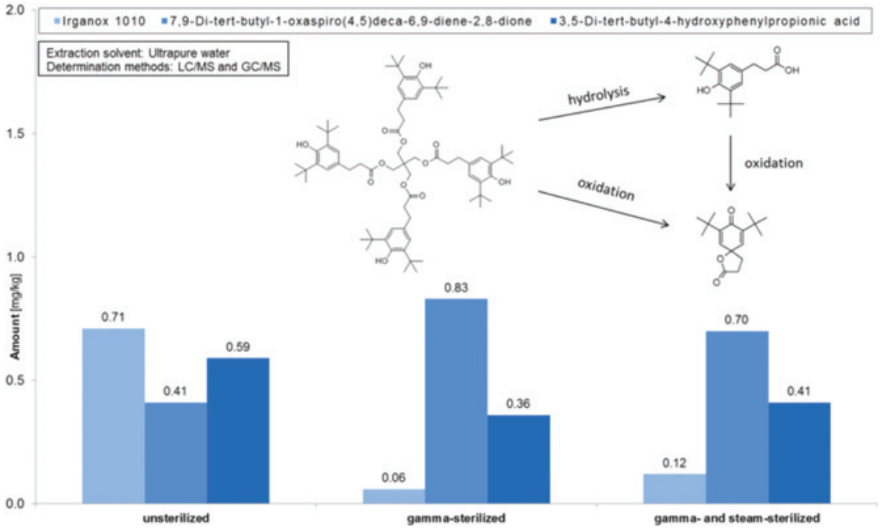


FIGURE 4: Concentration of water-extractable Irganox 1010 and degradation products out of sample B after different sterilization procedures found by LC/MS or GC/MS (depending on analyte).

General observations

- The total content of organic components (VOC, SVOC, and NVOC) deviates quite significantly between various bromobutyl stoppers from different manufacturers.
- In most cases, acetone (residual solvent or rubber oxidation product) and bromomethane (radical rubber degradation product) can be found prior to any sterilization. Their concentration significantly increases upon gamma-sterilization (Fig. 2).
- The total amount of SVOC from samples A – E strongly depends on the applied extraction solvent and method. Concerning extraction of rubber oligomers, rubber degradation products and antioxidants extraction in dichloromethane is most efficient. In most cases, extraction of fatty acids—e.g. from palmitic or stearic acid derivates—seems to be more efficient using isopropanol—probably depending on kind of derivate (e.g. zinc stearate, etc.) contained in rubber formulation.
- A steric hindered phenolic antioxidant can be found in all stoppers—e.g. BHT (sample A, D, E), Irganox® 1010 (sample B) or Irganox® 1076 (sample C). Extraction of these antioxidants was most pronounced using dichloromethane as extraction solvent (Fig. 3).

Observations demonstrating the impact of sterilization

- Sum of VOC out of all samples significantly increases upon gamma-sterilization.
- Significant amounts of isobutene and other unsaturated hydrocarbons (e.g. rubber pyrolysis products) out of all stoppers can be observed only after gamma-sterilization (Fig. 1-2).
- Generally, the sum of VOC decreases upon steam-sterilization of unsterilized and gamma-sterilized stoppers. That effect is also observed for single substances like isobutene, bromomethane, and acetone. (Fig. 2)
- The concentration of extractable antioxidants out of all samples significantly decreases upon gamma-sterilization (loss of 50 % and more). Steam-sterilization had no significant influence on antioxidant concentration. In some cases, a slight tendency for an increasing concentration of extractable antioxidants can be estimated (Fig 3).
- Extractable heptadecane—as well as some other saturated and unsaturated hydrocarbons—out of all samples can be found only upon gamma-sterilization and gamma—with additional steam-sterilization (Tab. 1).
- In general, the total amount of extractable palmitic and stearic acid (fatty acids) out of all stopper types increased upon gamma-irradiation. In most cases a more or less pronounced decrease of extractable fatty acids can be observed after steam-sterilization of unsterilized and gamma-sterilized. This observation was strongest for extracts in DCM and ultrapure water. The use of ultrapure water seems to result in extraction of (most probably) surface-orientated fatty acids, rather than extraction out of the depth of the material.
- After gamma-sterilization of sample B, the concentration of water-extractable 7,9-di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione—one possible Irganox® 1010 degradation product—increases. This substance should be estimated as a potential Leachable, especially into aqueous drug formulations. (Fig. 4).
- A significant influence or trend of sterilization on the concentration of extractable cyclic rubber oligomers—e.g. C13H24 and C21H40—was not observed for any examined sample (Tab. 2).

As exemplary shown in Figure 1 (only VOC), compounds were found in different amounts depending on the sterilization method. In a subsequent toxicological evaluation, any of those Extractables would be assessed by reviewing available toxicological data and coming up with a realistic PDE limit based on exposure routes (oral, inhalation, dermal, ophthalmic, injection). To show the general procedure acetone can be

taken as an example, defined by USP <467> as a Class 3 residual solvent[16], the PDE is limited to not more than 50 mg per day; the maximum amount after gamma sterilization found in this study was 2.5 µg / stopper, which demonstrate that acetone is not critical in this case. Applying the same procedure onto other extractables (e.g. bromo organic substances) may generate a different risk profile.

CONCLUSION

The results of the study clearly demonstrate that sterilization of polymer components influences the respective extraction profile quite significantly. Thus, we can expect that the amount and the kind of leachables from a primary packaging product that comes into contact with a pharmaceutical formulation also depends on the sterilization processing.

It was observed that some of the stopper additives, like antioxidants, were degraded upon gamma sterilization. This might influence the stability of the base polymer material and lead to formation of pyrolysis or radical degradation products of the basic polymer, e.g. formation of isobutene or bromomethane. Conversely, the steam sterilization seems to have a "cleaning" effect with regards to VOC, some SVOC and fatty acids.

While this kind of study is useful for giving trends, it is too general to fulfill the specific needs of an individual drug product/container system. Therefore, customized E&L investigations, considering the drug composition and processing as well as the packaging properties, should be executed on the basis of best practices extractables guidelines. **CP**

References

1. *US Government Printing Office, Equipment Construction, "Code of Federal Regulations, Food and Drugs," Title 21, Part 211.65.*
2. *FDA Guidance for Industry "Container Closure Systems for Packaging Human Drugs and Biologics," May 1999.*
3. *FDA Guidance for Industry "Nasal Spray and Inhalation Solution, Suspension and Spray Drug Products," July 2002.*
4. *Draft FDA Guidance for Industry "Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products," October 1998.*
5. *European Commission, "Good Manufacturing Practices, Medicinal Products for Human and Veterinary Use," Volume 4, 1998.*
6. *USP <1663> Assessment of Extractables associated with Pharmaceutical Packaging/Delivery Systems*
7. *USP <1664> Assessement of Drug Product Leachables associated with Pharmaceutical Packaging/Delivery Systems*
8. *EMEA Guideline on "Plastic Immediate Packaging Materials," December 2005.*
9. *BPSA Extractables and Leachables Subcommittee, "Recommendations for Extractables and Leachables Testing, Part Two: Executing a Program," BioProcess Int. 6(1) 2008: 44-52.*
10. *Norwood DL, Paskiet D, Ruberto M, et al., "Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products: An Overview of the PQRI Recommendations," Pharmaceutical Res. 2008; 25(4):727-739.*
11. *Scott Keith "Extractables and Leachables Testing of Polymer Device Components" 2007 Proc. Pharm. Polym. Paper 12, June 20-21 Basel Switzerland.*
12. *Ball D, Blanchard J, Jacobson-Kram D, et al. "Development of Safety Qualification Thresholds and Their Use in Orally Inhaled and Nasal Drug Product Evaluation," Toxicol Sci. 2007; 97(2):226-236.*
13. *Paskiet D, Jenke D, Ball D, Houston C, Norwood D, Markovic I. "The Product Quality Research Institute (PQRI) Leachables and Extractables Working Group Initiatives for Parenteral and Opthalmic Drug Product (PODP)" PDA J. Pharm. Sci. Technol. 2013, 67(5), 430-447.*
14. *Jenke D, Castner J, Egert T, Feinberg T, Hendriker A, Houston C, Hunt DG, Lynch M, Shaw A, Nicholas K, Norwood DL, Paskiet D, Ruberto M, Smith EJ, Holcomb F. "Extractables Characterization for Five Materials of Construction Representative of Packaging Systems Used for Parenteral and Opthalmic Drug Products" PDA J. Pharm. Sci. Technol. 2013, 67(5), 448-511.*
15. *www.ich.org (accessed 2/6/2014).*
16. *http://www.usp.org/sites/default/files/usp_pdf/EN/USPNF/generalChapter467Current.pdf (accessed 2/6/2014).*