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Table 2. Results of Permeation/Leakage Tests Conducted Under this Program1.

Sample ID	Description	Amount Spiked (µg)	Total Amount Recovered (µg) from sample	% Recovery (relative to amount spiked)	Vapor Conc in 5 Liter bag (µg/L = mg/m³)	Percentage of PEL	Factor of PEL
O-HS 55-7 DF 100	Single Orange Bag, containing 5 mL THF, heat sealed below all the closures (this is the single bag-no seal control). Sampled after 24 hours. One and only test.	4445000	164	0.004	32.8	6	0.1
O-R1 55-4 DF 10000	Single Orange Bag containing 5 mL THF, folded and sealed at the interface of where the orange portion of the bag turns clear. Sampled after 24 hours. Replicate 1.	4445000	2335	0.053	467	79	0.8
O-R2 55-5 DF 10000	Single Orange Bag containing 5 mL THF, folded and sealed at the interface where the orange portion of the bag turns clear. Sampled after 24 hours. Replicate 2.	4445000	4810	0.108	962	163	1.6
O-R3 55-6 DF 10000	Single Orange Bag containing 5 mL THF, folded and sealed at the interface of where the orange portion of the bag turns clear. Sampled after 24 hours. Replicate 3.	4445000	11250	0.253	2250	381	3.8
ODB-R1 55-1	Orange Bag, doubly contained, inner bag containing 5 mL THF, each folded and sealed at the interface of where the orange portion of the bag turns clear. Sampled after 24 hours. Replicate 1.	4445000	0.62	<0.001%	0.124	0.02	0.0002
ODB-R2 55-2	Orange Bag, doubly contained, inner bag containing 5 mL THF, each folded and sealed at the interface of where the orange portion of the bag turns clear. Sampled after 24 hours. Replicate 2.	4445000	1.86	<0.001%	0.372	0.06	0.0006
ODB-R3 55-3 DF 100	Orange Bag, doubly contained, inner bag containing 5 mL THF, each folded and sealed at the interface of where the orange portion of the bag turns clear. Sampled after 24 hours. Replicate 3.	4445000	6.1	<0.001%	1.22	0.21	0.002
SB-DS 55-12 DF 10000	Double adhesive strip, single bag containing 5 mL THF, sealed and folded per LOKSAK instructions. Sampled after 24 hours. One and only test.	4445000	6150	0.138	1230	208	2.1
DB-DS 55-11 DF 100	Double adhesive strip, double bag arrangement with inner bag containing 5 mL THF, sealed and folded per LOKSAK instructions. Sampled after 24 hours. One and only test.	4445000	11.5	<0.001%	2.3	0.39	0.004

NOTE: Density THF =0.889 g/mL at 25 °C. µg = micrograms; L = liter; mg = milligram; m^3 = cubic meter. PEL=OSHA permissible exposure limit (8 hour time weighted average) for THF = 590 mg/m^3.

Please note MRI did a one bag permeation (PEL) test.

Then MRI Global did a second test with a CBRNSAK placed inside another CBRNSAK. Please note how the PEL on the highlighted 2 bag test, was reduced by about 1,000 %.

LOKSAK has added a third larger CBRNSAK for a three-bag protection and the PEL vanished.

Based on what you plan to store, you will have a choice of how much PEL is necessary for your application.

We are adding more sizes to create back up bags to always give our customers various options.

Samples are available upon request.

Entire Report is attached.

Permeation Testing of Two LOKSAK, Inc. Sampling Bags

Final Technical Report

April 20, 2018

MRIGlobal Project No. 311532

FOR:

LOKSAK, Inc.

Linda Kennedy, President
P.O. Box 7127
Naples, Florida 34101

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April 20, 2018

LOKSAK, Inc.
P.O. Box 7127
Naples, FL 34101
Attn: Ms. Linda Kennedy
President

Subject: MRIGlobal Final Report entitled, "Permeation Testing of the LOKSAK, Inc. FORENSAK"

Dear Ms. Kennedy:

MRIGlobal is pleased to submit this Final Report, "Permeation Testing of the LOKSAK, Inc. FORENSAK" based on the tests authorized in the MRIGlobal Proposal dated February 22, 2018 (Proposal No. 829274). The tests conducted and reported herein were modified and approved by LOKSAK, Inc. via several teleconferences and emails.

Section 1.
Objectives and Program Tests

The objective of this program was to determine, quantitatively, the leakage/permeation of THF sealed within two types of LOKSAK bags under two separate experimental regimes. The regimes included either single bag testing or double bag testing described fully below.

One bag type tested (a modified OPSAK) had a single adhesive strip along with a standard, single tongue and groove closure system. The bags were clear, the closure was orange in color and, throughout the remainder of this report, these bags will be referred to as "orange bags." The second style of bag, also a modified OPSAK, had two adhesive strips with a standard, single tongue and groove closure (black in color), referred to throughout as the "double adhesive" bag. Photographs of each type of bag tested are included in Figure1. LOKSAK, Inc. supplied each type of bag with detailed instructions as to the proper way to seal the bag.

MRIGlobal tested the permeation/leakage of each bag type against tetrahydrofuran (THF) using a single and double bag ensemble. The single bag ensemble was simply THF added to the bag and sealed per LOKSAK instructions. The double bag ensemble was THF was added to one of the bags (as just described), sealed appropriately, then this bag was placed inside a second bag of the same type and sealed appropriately. All ensembles were allowed to permeate under ambient conditions (no adjustment of temperature or humidity) inside a 10 liter (L) Tedlar bag filled with 5 L of nitrogen. Complete details of the testing and ensemble preparation is below.

Figure 1. Bags Tested Under this Program
The "Orange Bag" is on the left and the "Double Adhesive Bag" is on the right

All the tests conducted on the bags as described are summarized in Table 1.

Table 1. Description of Permeation/Leakage Tests Conducted Under this Program

Bag Type and Adhesive Type	Ensemble Type	Replicates	Test Summary
Modified OPSAK—Single Adhesive Strip with an Orange Closure "Orange Bag"	Single-bag. Heat Sealed	1	Using the Orange Bag, a single bag leakage test using THF as the target was performed. The bag was heat sealed at MRIGlobal below all the closures and not inserted inside a second bag (this is the single bag-no seal control test). See Figure 2 for heat seal location.
Modified OPSAK—Single Adhesive Strip with an Orange Closure "Orange Bag"	Single-bag	3	Using the Orange Bags, a single bag leakage test using THF as the target was performed. MRIGlobal, per LOKSAK instructions, folded and sealed this bag right at the interface of where the orange portion of the bag turns clear. This test was performed in triplicate.
Modified OPSAK—Single Adhesive Strip with an Orange Closure "Orange Bag"	Double-bag	3	Using the Orange Bag a double bag leakage test using THF was performed. MRIGlobal, per LOKSAK instructions, folded and sealed this bag right at the interface of where the orange portion of the bag turns clear, then placed the THF loaded bag inside a second Orange Bag and sealed as above. This test was performed in triplicate.
Modified OPSAK—Double Adhesive Strip with a Black Closure "Double Adhesive Bag"	Single-bag	1	Using the double adhesive bag a single bag leakage test using THF as the target was performed. MRIGlobal folded and sealed this bag per LOKSAK instructions. This bag was not double bagged. This test was performed only once.
Modified OPSAK—Double Adhesive Strip with a Black Closure "Double Adhesive Bag"	Double-bag	1	Using the double adhesive bag a double bag leakage test using THF as the target was performed. MRIGlobal folded and sealed the bag per LOKSAK instructions, then placed the THF loaded bag inside a second double adhesive bag and sealed as above. This test was performed only once.

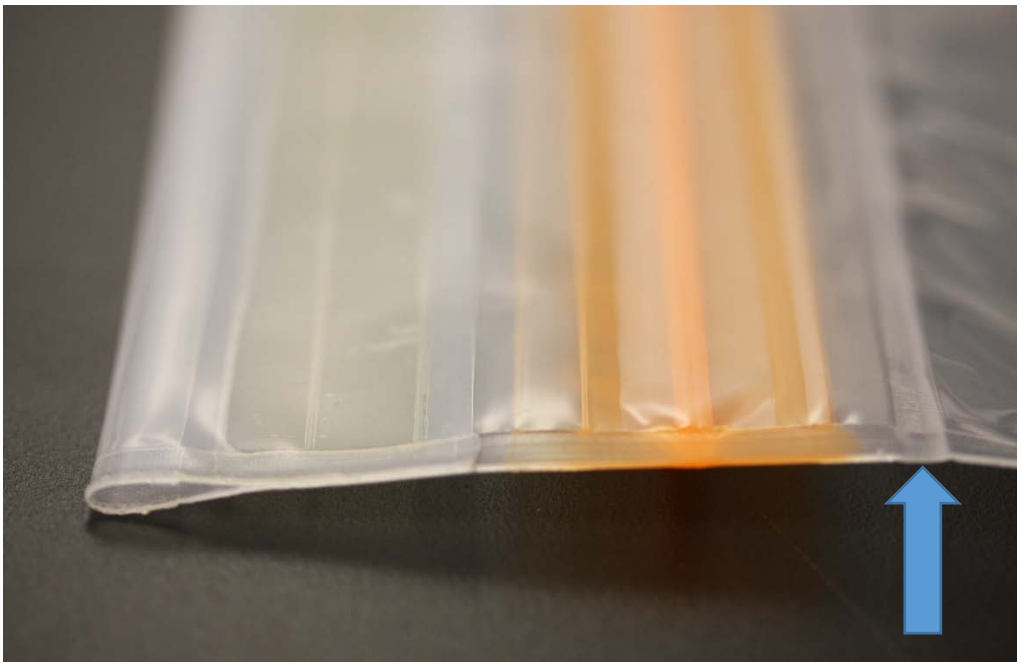

Figure 2. Heat Seal Location (arrow) for the Single-Bag-Heat Seal Permeation/Leakage Test

Section 2.
Results

The test results for the experiments described in Table 1 are summarized in Table 2. All data and results in Table 2 have been reviewed and are final. Experimental details describing how this data was collected is included in Section 3, Experimental.

Table 2. Results of Permeation/Leakage Tests Conducted Under this Program1.

Sample ID	Description	Amount Spiked (µg)	Total Amount Recovered (µg) from sample	% Recovery (relative to amount spiked)	Vapor Conc in 5 Liter bag (µg/L = mg/m^3)	Percentage of PEL	Factor of PEL
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NOTE: Density THF =0.889 g/mL at 25 °C. µg = micrograms; L = liter; mg = milligram; m^3 = cubic meter. PEL=OSHA permissible exposure limit (8 hour time weighted average) for THF = 590 mg/m^3.

The amount of THF spiked into a bag was 5 milliliters (mL) for all tests and accounting for density and converting to micrograms, this is 4,445,000 µg. The "Total Amount Recovered (µg) from sample" is the amount of THF found in the five liters nitrogen inside the Tedlar bag containing the test ensemble. All five liters of volume was sampled in all tests. The "% Recovery" is the THF quantitated in the five-liter Tedlar bag relative to the 5 mL of THF contained within the test ensemble. The last two columns represent the amount of THF quantitated in the five-liter Tedlar bag relative to the Occupational Safety and Health Administration's (OSHA) permissible exposure limit (PEL) which for THF is 590 mg/m3. The PEL is an 8-hour time weighted average promulgated by OSHA for use a guide for determining maximum worker exposures. The PEL information presented is strictly for comparison purposes amongst the data in table. Samples were diluted as necessary to obtain results within the calibration range of the instrument.

All permeation/leakage samples and quality control samples were analyzed using gas chromatography with flame ionization detection (GC-FID) which was calibrated for quantitative THF analysis before use. A complete description of this method can found in Section 3. The instrument calibration and quality control samples employed and associated results for this study are summarized in Table 3. The initial calibration passed all data quality objectives (DQO) and was used for quantitation of all test run samples. The method blank, method spike and Positive Control Sample (PCS) all passed DQO criteria.

There were eight instrument blanks analyzed during the analytical sequence which consisted of a total of fifty separate injections on the GC-FID. The analytical sequence was prepared in the following order: the calibration samples were injected first, then method blanks, the matrix spike, the PCS and then the test run samples in order from the most dilute to the most concentrated. Interspersed throughout this sequence, after approximately every ten injections on the instrument, were instrument blanks (eight in all) continuing calibration verification (CCV) samples (eight in all). One instrument blank and one CCV failed DQO criteria. These two quality control samples were back to back and were preceded by six injections of test run samples that contained very large amounts of THF. These samples were undiluted extracts from the single bag ensembles. These two quality control sample failures were the result of THF carry-over from those six high THF-concentration samples. This conclusion is based on the fact that the two samples after these two failed quality control samples were a CCV and another blank. The CCV passed DQO criteria at 110% recovery, which was immediately followed by an instrument blank injection which showed no trace of THF.

These two quality control sample DQO failures had no impact on the results reported herein since none of the analyses before these were reportable results since those samples greatly exceeded the calibration range of the instrument. The diluted results for those samples are reported and they were all bracketed by blanks and CCV's that met DQO criteria.

Table 3. Calibration and Permeation Data Quality Samples Objectives and Results

Quality Control Procedure	Frequency	Data Quality Objective	Result
Initial Calibration (ICAL) Minimum four levels	Before sample analysis. Repeat when analytical conditions change.	$R^2 \geq 0.99$ Low-level standard must provide a response that is ≥ three times the noise level. Percent accuracy of each initial calibration standard must provide 70%-130% accuracy of the true concentration.	A five-point calibration was used with a linear correlation coefficient (R^2) of 0.9997. The lowest standard was greater than three times the noise level. The percent accuracy of the initial calibration standards were all between 96% and 117%.
Instrument Blank	Before ICAL. Immediately after high-level ICAL standard. Before sample analysis and a blank run approximately after every ten samples.	No peak in target retention time window with a response that is greater than the low-level standard.	All blanks were free of target except one, which was analyzed after six consecutive samples containing high levels of THF which caused carry-over. This contaminated blank had no effect on the reported data. See text for further discussion.
Continuing Calibration Verification (CCV)	At the end of each day of sample analysis following an ICAL. At the beginning and end of each day of sample analysis.	70%-130% accuracy	Eight CCV's were analyzed during the analytical sequence. Seven met DQO ranging from 100% to 129% accuracy. The one failed CCV occurred after seven consecutive samples containing high levels of THF, which resulted in carry-over, and a high level of THF quantitated in this CCV. This failed CCV had no effect on the reported data. See text for further discussion.
Method Blank (empty Tedlar bag filled with nitrogen)	One during every permeation set.	No chemical target or interference observed in the retention time window of the target chemical.	Method blank was clean showing no THF above the detection limit.
Method Spike	One performed prior to permeation testing.	Neat liquid spike directly on sorbent tube with a target recovery of 55% to 125% with a goal of 80% or greater.	Method spike sample gave 107% recovery meeting DQO.
Positive Control Sample	One to be performed with each permeation set.	Neat liquid spike directly into a Tedlar bag containing 5 liters of nitrogen. Target recovery of 55-125% with a goal of 80% or greater.	Positive Control Sample gave 103% recovery meeting DQO.

Section 3.
Experimental

3.1 Ensemble Configuration

The permeation/leakage testing was executed as follows. A gauze pad was placed inside a candidate bag and spiked with 5 mL of THF. The candidate bag was then sealed per LOKSAK instructions. If the test was a single bag ensemble test, then the THF spiked bag was bag was placed inside a ten-liter Tedlar bag. The Tedlar bag was then filled with 5 liters of nitrogen and the ensemble was allowed to permeate for a 24-hour period. If the test was a double bag ensemble, the THF spiked bag was then carefully placed in a secondary bag of the same type and sealed. The double bag ensemble was placed inside a ten-liter Tedlar bag. The Tedlar bag was filled with 5 liters of nitrogen and the ensemble was allowed to permeate for a 24-hour period.

Following the permeation event, the headspace of the Tedlar bag was collected using a solid sorbent tube. After the collection event, the sorbent tubes are solvent extracted and submitted for analysis. Figure 3 is a photograph of the entire configuration with a double-bag ensemble inside a 10 liter Tedlar bag.

Figure 3. Testing Configuration

3.2 Sample Collection of Organic Solvents

MRIGlobal identified and validated sorbent-based method for collection of gas phase THF. The method (OSHA Method #111 and described in Microchemical J., 101, 2012, 37-42) employs glass sorbent tubes packed with 150 mg of ANASORB (coconut shell charcoal, SKC, Inc.) to "sample" headspace at a flow rate of 50 mL/min. This collection methodology was developed under previous programs at MRIGlobal. Tubes collecting THF were extracted with carbon disulfide (CS2). The sorbent-based collection is very reproducible as the quality control results in Table 3 indicate.

3.3 Sample Analysis by GC-FID

MRIGlobal analyzed the extracted solid sorbent tubes according to "A Capillary Gas Chromatographic Procedure for the Analysis of Nine Common Residual Solvents in Water-Insoluble Bulk Pharmaceuticals", J. Chrom. Sci., 36, March 1998. Gas chromatography with flame ionization detector (GC-FID) easily accommodated analysis of THF by chromatographically separating the dilution solvent, in this case CS2. Table 4 summarizes the acquisition parameters for the analysis using an Agilent GC-FID 5890N.

Table 4. GC-FID Analysis Parameters

Column:	Rtx-1301
length:	30 m
diameter:	.53 mm
thickness:	3 µm
initial temp	45°C
initial hold:	8 min
ramp 1:	10°C/min
hold:	195°C
final hold:	5 min.
total run time:	28 min.
inlet temp.:	200°C
inlet type:	Splitless
total flow rate:	60 mL/min
injection volume:	1 µm
carrier gas:	Helium
mode:	constant flow
column flow rate:	3.2 mL/min
FID temp:	260°C
FID hydrogen flow rate:	30 mL/min
FID air flow rate:	400 mL/min
Helium make-up gas:	30 mL/min

3.4 Test Procedures

3.4.1 Set-Up of THF Containing Bag Ensembles

1. Obtain and label bags to be tested.
2. Place gauze pad inside test bag. The gauze pad was large enough to ensure five (5) mL of THF will soak into it without a significant amount of free-flowing liquid observable inside the bag.
3. Spike the gauze pad with five (5) mL THF without contaminating the outside of the bag or the zippered closure area.
4. Seal the bag closure per LOKSAK instructions. If this was a single bag ensemble test, Step 5 was conducted. For a double bag ensemble test, the THF spiked bag was placed into a secondary test bag of the same type and sealed per LOKSAK instructions.
5. Place the test bag ensemble inside the 10-liter (10 L) Tedlar bag and heat seal the Tedlar bag.
6. Using a calibrated mass flow controller, deliver five (5) liters of ultra-high purity (UHP) Nitrogen (one L/min for five minutes).
7. Start the timer and let the test proceed for twenty-four (24) hours.

8. Using a vacuum pump, set the sorbent tube to a designated flow rate of 50 mL/min and record the starting flow.
9. At the end of the test period, connect the sorbent tube to the ¼" port on the Tedlar bag and collect the headspace for approximately 100 minutes.
10. Verify and record the ending flow of the sorbent tube.
11. Store the sorbent tube in the freezer or extract as described below.

3.4.2 Sorbent Tube Extraction for THF

1. Score the inlet end of the tube using a file and snap off the end.
2. Using a cleaned copper wire plunger on the outlet end, push the sorbent bed onto a piece of weighing paper.
3. Using clean forceps, separate the glass wool and foam plugs from the sorbent. Discard the glass wool and foam plugs.
4. Carefully transfer the sorbent into a clean, tapered glass vial.
5. Add 0.5 mL of CS_2 using a calibrated pipettor or syringe.
6. Sonicate the samples for thirty (30) minutes. Record start and end time.
7. Using a disposable glass pipette, transfer the extract to a three (3) mL plastic syringe affixed with a 0.2-µm Teflon acrodisc.
8. Filter the extract into a glass GC vial. Aliquot as appropriate.
9. Submit for GC/FID analysis.

Section 4.
Observations and Discussion

Inspection of Table 2 shows that “double bagging” significantly reduces the amount of THF permeating from the ensemble. The amount of reduction is approximately the same for the single adhesive strip versus the double adhesive strip bag type.

Regarding the single bag testing, all data should be compared to the single bag-heat sealed control, which permeated 164 micrograms of THF after 24 hours (row 2 of Table 2). This represents the permeation rate through the body of the bags completely independent of the closure type. The permeation of THF was remarkably consistent between the single adhesive closure (orange bag) and double adhesive closure (double adhesive) bag. The average of the three single orange bag test runs was 6131 micrograms of THF (average of rows 3-5 in Table 2). The single ensemble double adhesive bag result was 6150 micrograms of THF. These values are 37-times higher than the heat-sealed control indicating the majority of the THF permeation is via the closure. Moreover, single or double adhesive (with latter requiring three folds to seal) did alter the permeation/leakage over the 24-hour period.

Photographs of each type of bag, after sealing per LOKSAK instructions are displayed in Figures 4 and 5. Note in Figure 4, for the single adhesive bag, a loop forms after applying the adhesive strip to the body of the bag. MRIGlobal staff attempted to minimize that loop, but it formed in all cases because of the flexibility of the OPSAK material and stiffness of the tongue and groove closure (the orange part of this bag). Thus, it is possible to surmise that the THF readily permeates through the tongue and groove closure and out of the bag through this loop. The tongue and groove closure of this orange bag is made of polyethylene (per communications from LOKSAK) and in a previous study conducted by MRIGlobal, it was found that polyethylene is readily permeated by THF at ambient conditions.

The double adhesive strip bag (single ensemble test) as stated above displayed nearly identical THF permeation even with the much more apparent tighter closure system (three folds, two adhesives). As shown in Figure 5, this “end on” photograph shows there is no visual large “loop” like the single adhesive bag developed upon closure, but there are small ones. MRIGlobal staff attempted to fold this double adhesive bag as tightly as possible for all test runs, but the small gaps could not be eliminated.

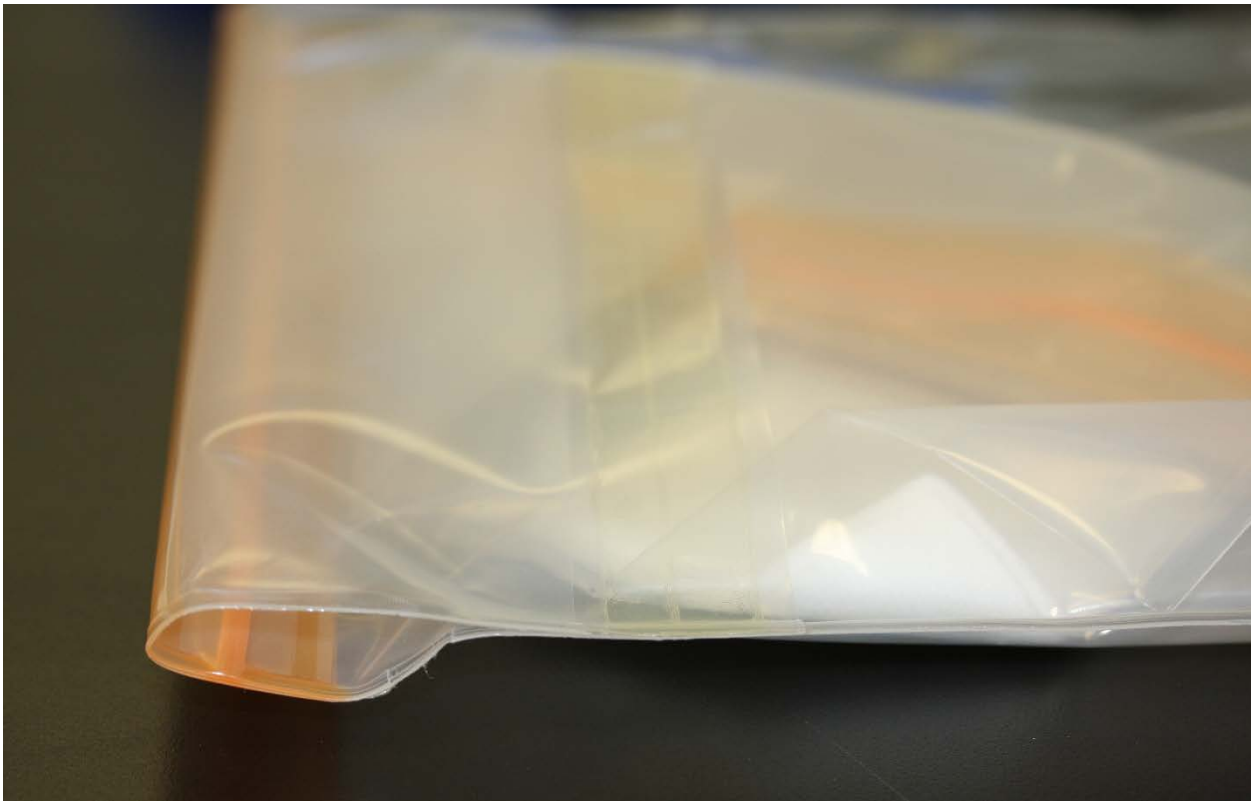

Figure 4. Photograph of the Single Adhesive Bag Type After Closure

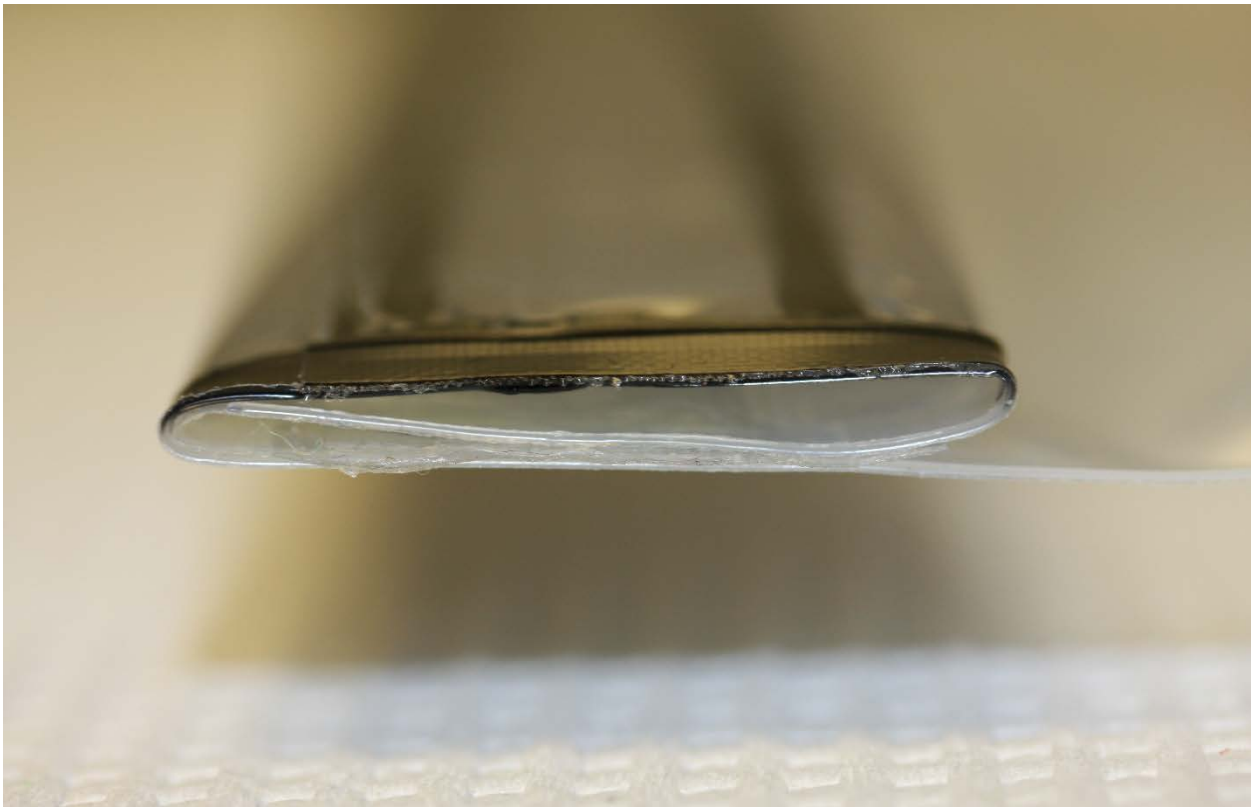

Figure 5. "End-On" Photograph of the Double Adhesive Bag Type After Closure

The test results reported herein cannot, unambiguously, determine the source of the leakage/permeation of THF through the tongue and groove closure. In other words, through micro-channels in the tongue and groove (see Figure 6) or straightforward permeation through the all areas of polyethylene closure. Since the OPSAK material (EVOH) is on the outside of the closure, this strongly indicates the permeation is coming through the actual tongue and groove system (see Figure 6).

If this assumption is true, possible ways to minimize this permeation of THF through this type of closure include (note these are not recommendations but only observations that may minimize leakage/permeation):

1. Fabricate the closure(s) from a less-permeable material than polyethylene.
2. Employ a double closure tongue and groove system as shown in Figure 6.
3. If adhesive strips are used, one strip positioned directly above the closure would potentially prevent a gap of material when it is folded over. A second adhesive strip positioned directly on the back of the first fold would then, potentially, result in two tight adhesions with little to no material on the sides for leakage.
4. If the bags are used for first responders and sampling of forensic samples, selling them in a two-bag kit, would, as shown by the data, herein, provide excellent permeation/leakage resistance. The one bag where the sample would be placed needs to be smaller than the outer, second bag, for easy insertion. MRIGlobal staff had to fold and bend in the corners to prepare the double bag ensembles for this study.

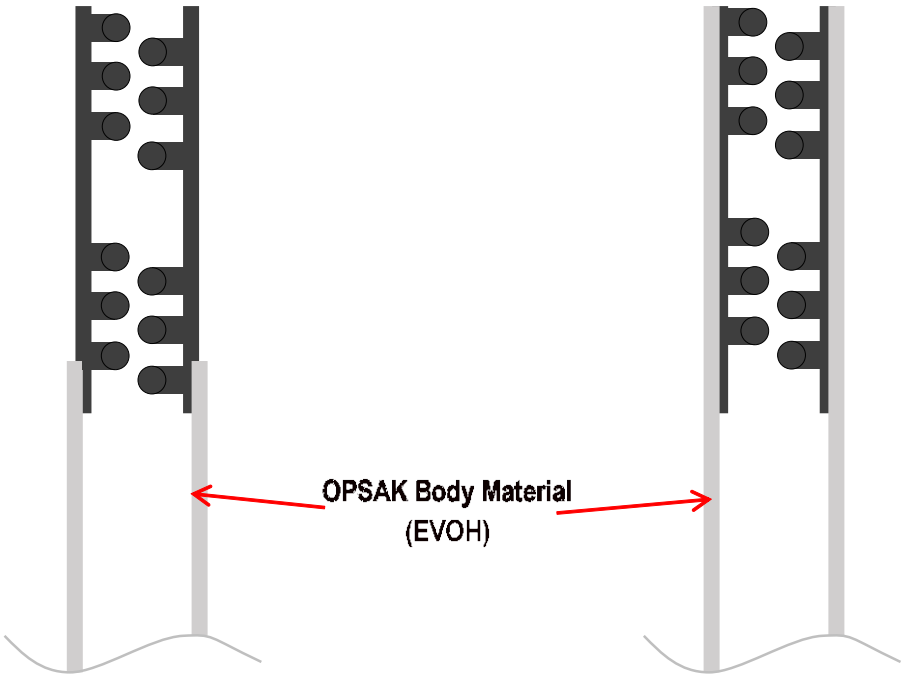

Figure 6. Left – Schematic of a tongue and groove closure without EVOH material covering the closure. Right – Schematic showing EVOH extended to cover the closures. EVOH is light gray, polyethylene (PE) is black.