



Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Biologics Evaluation and Research

Pharmacology/Toxicology Review
Division of Hematology
Office of Blood Research & Review

To: NDA BN110059 and NDA BN110059/014 (x-ref IND 14199 and Master File (b)(4))

Reviewer: M. Keith Wyatt, Ph. D., Pharmacologist, CBER\OBRR\DH

Through: Anne M. Pilaro, Ph.D., Supervisory Toxicologist,
CBER\OBRR\DH

Applicant: Hemerus, St. Paul, MN

Product: Hemerus Leukocyte Reduction System with SOLX[®]

Purpose: Hemerus responses to FDA Information request (IR) sent June, 2012

Date received: July 26, 2012

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Recommendation:

Thrombocytopenia was observed in rats administered leachate from the circuits and storage bags that comprise the SOLX[®] system, and remains a safety concern that has not been adequately addressed by the Applicant. Therefore, the Pharmacology/Toxicology discipline recommends that NDA BN110059 be issued a complete review (CR) letter, and that the Applicant be required to perform a risk assessment based on results from the previous extraction study, # 06-5803-N2, conducted on an earlier version of the Applicant's leukocyte reduction system. Results from the risk assessment will ensure the safety of blood components produced using the SOLX[®] system that may contain these leachates.

The Applicant will also be required to identify any significant differences between the earlier, HRC-600-C Leukocyte Reduction Filtration System for Red Cell (BK070024 8/24/2007) system and the new SOLX[®] system, to ensure the relevancy of results from the previous extraction study to the safety of blood components produced with the new SOLX[®] system.

Letter-ready comments #1, #2 and #3 should be transmitted directly to the Applicant:

1. Regarding extraction studies on SOLX[®] circuits

The responses you provided in Amendment 14 to BN110059 for FDA questions #16, 21b and 24a are not adequate to address the risk associated with the thrombocytopenia observed in rats administered leachate from the circuits and storage bags that comprise the SOLX[®] system. This issue remains a safety concern that must be addressed prior to approval.

To ensure the safety of the blood components produced using the SOLX[®] system that potentially contain these leachates, provide a risk assessment based on results from the previous extraction study # 06-5803-N2, conducted on an earlier version of your leukocyte reduction system. Identify any significant differences between the earlier, HRC-600-C Leukocyte Reduction Filtration System for Red Cell (BK070024 8/24/2007) system, and the new SOLX[®] system to ensure the relevancy of the results from the previous extraction study to the safety of products produced with the SOLX[®] system.

Please be aware you may be required to perform an additional leachables and extractables study and a separate risk assessment for the SOLX[®] system, if any significant differences are identified between the earlier HRC-600-C Leukocyte Reduction Filtration System for Red Cell (BK070024 8/24/2007) system and the new SOLX[®] system.

2. Regarding the Agar diffusion study performed by --(b)(4)----, 12-3101-G1

- a) Please identify the names and manufacturers of the 3 different inks printed on the label strips that were evaluated during the Agar diffusion study.

3. Regarding label inks from ---(b)(4)-----

- a) Please confirm the ribbon in-house print ink ---(b)(4)----- and ---(b)(4)---- manufactured by -----(b)(4)-----, respectively, will only be used on labels applied to packaging and carton material used to transport the SOLX[®] system.

Additional comments on information presented in Master File (b)(4) regarding the inks and label stocks used on SOLX[®] blood bags will be submitted directly to the manufacturer and holder of the Master File, JMS-Singapore.

Introduction

Hemerus (the Applicant) has submitted responses to an information request (IR) sent in June, 2012 regarding the adequacy of data submitted in their NDA to support approval of the SOLX[®] Leukocyte Reduction System. The specific pre-clinical toxicology questions sent to the Applicant in the June, 2012 IR letter focused primarily on results from (1) biocompatibility testing of leachates from storage bags and circuits that comprise the SOLX[®] system; (2) integrity and stability testing of print inks and; (3) cytotoxicity testing conducted on print inks used on blood bag labels.

In Amendment 14 to the NDA submission, the Applicant has provided responses to the June, 2012 IR generated by FDA following mid-cycle review; the initial FDA comments for each IR are provided (in italics), below. Based on the new results from the additional studies conducted, the Applicant has replied to each IR; the Applicant's response is summarized below each IR, or in tables or text excerpted from the Applicant's IR reply submitted on July 26, 2012. FDA follow-up comments to the Applicant IR responses are presented in red text.

A detailed review of the data contained in the Applicant's final study reports submitted to address the IR begins on page 13 of this memo.

Applicant's reply to questions in IR sent June 2012

Pharmacology / Toxicology:

13. *Regarding label stocks from (b)(4):*

The composition of the adhesive, called (b)(4), used on label stocks manufactured by (b)(4) was described as an acrylic, but the full formulation of the material was not provided and should be submitted as an amendment to MF3 (b)(4)

Hemerus Response:

JMS Singapore has received additional information from the supplier and acknowledged they will update Biologics Master File # (b)(4) by the end of July 2012.

FDA evaluation of the Applicant's response, August 2012: Based on review of the data submitted to MF (b)(4), the information provided regarding the adhesive used on label stocks manufactured by (b)(4) is adequate to ensure safety under the proposed conditions of use.

14. *Regarding the formulation for acrylate ink manufactured by (b)(4)*

- a. *Results previously provided in Report TPI119/PED/2009, submitted in the original NDA BN110059/0, suggest labels printed with acrylate ink maintain their integrity and stability under a variety of conditions and are therefore fully polymerized. While these previous data are acceptable, the cytotoxic potential of labels printed with acrylate ink manufactured by (b)(4) should be determined using the MEM elution assay <USP 87>.*
-

- b. Please confirm that (b)(4) will be used to polymerize acrylate ink to label stocks and also provide the name and location of the vendor responsible for performing the polymerization procedure.

Hemerus Response:

- a. Hemerus performed previous cytotoxicity testing on labels incorporating the (b)(4) ink. This information was presented in BN110059 Amendment 008 dated 3/7/12 (Appendix 2 12-0603 G1 Agar Diffusion Test ISO). The Agar Diffusion Test was conducted for labels of (b)(4). No biological reactivity (Grade 0) was observed in the (b)(4) mammalian cells at 48 hours post exposure to the test article.

Testing on the (b)(4) ink was also repeated as part of label testing recently performed. Passing results were repeatedly demonstrated for SOLX[®] System labels printed with (b)(4) ink (see (b)(4) Report 12-3101-G1 attached as **Appendix 4**).

- b. Yes, (b)(4) will be used to polymerize acrylate ink to label stocks. The supplier is referenced in Biologic Master File (b)(4) page 56. JMSS will update MF (b)(4) to highlight the role of the supplier to include printing, (b)(4) ink curing and die cutting.

FDA evaluation of the Applicant's response, August 2012: Results from the Agar diffusion study are acceptable, and demonstrate that the inks used on the SOLX[®] blood bag labels are not cytotoxic to mammalian cells. Polymerization using (b)(4) is an appropriate method for immobilizing acrylate-based inks, and is acceptable.

15. Regarding ribbon ink from (b)(4) please provide the following for review:

- a. The formulation for ribbon ink, called (b)(4) manufactured by Armor in (b)(4). This was not provided in (b)(4).
- b. An assessment of the potential cytotoxicity of ribbon ink (b)(4) used on Hemerus blood bag labels using the MEM Elution assay <USP 87>.
- c. The name and location of the printer responsible for applying ribbon ink (b)(4).
- d. The results from Ames assays performed by (b)(4).

Hemerus Response:

- a. JMS Singapore has acknowledged they will update Biologics Master File # (b)(4) the end of July 2012 with additional information.
- b. During previous cytotoxicity testing of printed labels, Hemerus inadvertently did not include labels incorporating the (b)(4) ribbon ink. For this reason, repeat cytotoxicity testing was performed on labels of SOLX[®] System Lot (b)(4) containing the (b)(4) ribbon ink. The ISO 10993-5 Agar Diffusion Test demonstrated that no biological reactivity (Grade 0) was observed in the (b)(4) mammalian cells at 48 hours post exposure to the test article. The testing results are documented in Toxikon Report 12-3101-G1 attached as **Appendix 4**.
- c. The name and location of the printer responsible for applying ribbon ink (b)(4) is:
JMS Singapore PTE LTD
440 Ang Mo Kio Industrial Park 1
Singapore 569620

-
- d. EC Directive EC 98/638 defines limits for heavy metals. JMS has requested the manufacturer to provide the information but it may take 6-8 weeks. Testing results for (b)(4) are summarized in JMSS Biological Master File (b)(4) pages 84-95. As a note, Ames testing (*S. Typhimurium* and *E. Coli* Reverse Mutation Assay) and heavy metal testing (Physicochemical Test for Plastics) were performed on the SOLX[®] System and reported in Original BN110059 Module 3.
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FDA evaluation of the Applicant's response, August 2012: As of August 17, 2012, FDA has not yet received the data from the Master File holder regarding the results from heavy metal analyses conducted on the inks manufactured by (b)(4). Results from an Ames assay referenced by the Applicant only apply to the potential genotoxicity of bag leachates, and not to potential mutagenicity associated with the print inks manufactured by (b)(4). This deficiency will be sent to the holder of the cross-referenced Master File to address.

Although the data provided show that the (b)(4) inks are stable when applied to label stock, suggesting human exposure and risk from the inks is low, the Applicant is still requested to submit any results from the Ames assay conducted on inks manufactured by (b)(4). This deficiency will be sent to the holder of the cross-referenced Master File to address.

Results from the Agar diffusion assay suggest print inks applied to label stocks are not cytotoxic to mammalian cells. These results are acceptable to ensure safety under the conditions of use.

16. Regarding additional toxicity test results reported by JMS:

The toxicity of PVC manufactured by JMS has not been evaluated by an in vivo implantation assay <USP 88>, or by direct contact and agar diffusion <USP 87> in vitro assays. Please submit results from these.

Hemerus Response:

It is Hemerus' opinion that PVC manufactured by JMS was adequately tested as part of an extensive in vitro and in vivo biocompatibility evaluation performed for the SOLX[®] System. The testing reported in BN110059 Module 4 was conducted using guidelines of ISO 10993 – *Biological Evaluation of Medical Devices* and FDA guidance document *Use of International Standard ISO-10993 Biological Evaluation of Medical Devices Part 1 Evaluation and Testing - May 1, 1995 (G95-1)*.

Additionally, the PVC manufactured at JMSS was used for manufacture of the previously cleared LEUKOSEP[®] HRC-600-C Leukocyte Reduction Filtration System for Red Cells (BK070024 8/24/2007).

FDA evaluation of the Applicant's response, August 2012: The response provided by the Applicant is not acceptable and does not adequately address the risk of leachable materials from SOLX[®] circuits and blood bags. Therefore, the Applicant will be requested to provide a risk assessment based on results from the previous extraction study (# 06-5803-N2) conducted according to procedures described in ISO 3826. The Applicant will also be asked to highlight any differences between the old leukocyte reduction system and the SOLX[®] system, to ensure the relevancy of the results from the previous extraction study to the safety of blood products produced with SOLX[®]. This deficiency will be communicated to the Applicant in the CR letter.

17. Regarding physicochemical testing:

- a. Please submit results on buffer capacity, residue contact on ignition and nonvolatile residue content in bag extracts.
- b. Please identify the components in PVC extracts used in the toxicity studies by mass spectrometry or at a minimum for total organic carbon (ToC) content.

Hemerus Response:

- a. Buffering capacity and nonvolatile residue content were reported in original NDA BN110059 Module 4 Appendix 4-16 Report 10-1868-G1 (*Physicochemical Test for Plastics-USP*) and met all criteria. The residue on ignition testing was not performed as the amount of nonvolatile residue did not exceed (b)(4)
 - b. USP testing demonstrated that nonvolatile residue of the SOLX[®] System extract was (b)(4) It is Hemerus' opinion that the request to test total organic carbon (ToC) is not required or necessary.
-

FDA evaluation of the Applicant's response, August 2012: The response provided by the Applicant is not acceptable. The Applicant will be requested to provide a risk assessment based on results from the previous extraction study (# 06-5803-N2) conducted according to procedures described in ISO 3826. The Applicant will also be asked to highlight any differences between the old Leukocyte reduction system and the SOLX[®] system to ensure the relevancy of the results from the previous extraction study to the safety of blood products produced with SOLX[®]. This deficiency will be communicated to the Applicant in the CR letter.

18. Regarding toxicity testing based on standards described in Japanese MHLW, please do the following:

-
- a. Clarify what the (b)(4) concentration of 50% means in the cytotoxicity results table. Describe the toxicity observed at concentrations above 50% using morphological and reactivity grades referenced in ISO 10993-5.
 - b. Indicate which of the following PVC pellets (b)(4) will be used to manufacture Hemerus bags.
 - c. Indicate the dose of PVC extract used in the acute toxicity studies.
 - d. Indicate the extract concentration used in the hemolysis and intracutaneous reactivity assays.

Hemerus Response:

- a. (b)(4) concentration of 50% means the sample extract is diluted with 50% of media which is used for cultivation. (b)(4) concentration of 100% means the sample extract without any dilution. Data from morphological and reactivity grade referenced in ISO 10993-5 is not available. However, the test results from quantitative method – Colony formation cytotoxicity test as referenced in ISO 10993-5 Annex B are provided.
- b. (b)(4)
 - Blood Bag
 - Not used
 - PVC Tubing
 - Blood Port
 - Y Connector

This information can be found in page 67 and 68 of the master file.

- c. PVC extract with concentration of 100% was used.
- d. PVC extract with concentration of 100% was used.

FDA evaluation of the Applicant's response, August 2012: The response provided by the Applicant is not acceptable. The results from a risk assessment (described in the FDA evaluation of the Applicant's responses to IR requests 16 and 17, above) should be provided, to address any safety concerns related to the extractable/leachable components of the SOLX[®] system. This deficiency will be communicated to the Applicant in the CR letter.

19. Regarding Resistance study, FR409400, March 6, 2012:

- a. Please justify why the stability of label inks was not evaluated against detergents, denatured alcohols, acids, and other solvents specified in ISO 2836 as exposure to these liquids may represent a worst case leaching scenario.
- b. Please confirm labels evaluated in the resistance study were printed with the ribbon ink (b)(4) (b)(4) manufactured by (b)(4) and with red and black acrylate inks manufactured by (b)(4) (b)(4). Please also confirm that label stocks used in this study were manufactured by (b)(4).
- c. The ink used to print the letter "T" on the label presented in Appendix A (located in the image on the right side at the bottom of page 13 of 17) was significantly reduced following treatment. Please quantify this reduction in ink intensity according to spectrophotometric method ISO 105-A03 referenced in ISO 2836.

Hemerus Response:

- a. As stated in Section 6 of Report FR409400, the agents chosen for the testing were those to which the labels were expected to be exposed in typical use and/or were representative of agents listed in the ISO 2836 standard. The liquid agents used for assessment were:

(b)(4)

- b. The labels evaluated in the resistance study FR409400 used label stock manufactured by (b)(4) and red and black acrylate inks manufactured by (b)(4). During our inquiry, it was discovered that the (b)(4) ink was not used to print the lot number.

Hemerus identified labels from SOLX[®] System lot (b)(4), using all the designated materials ((b)(4) label stock, red and black acrylate inks manufactured by (b)(4) and the (b)(4) ribbon ink), and repeated ink resistance testing. Protocol PC410930 and Report FR410930 describe the testing and are attached as **Appendices 5 and 6**. All testing criteria were met.

- c. The photos attached to Appendix A of Report FR409400 were intended for reference purposes only. All study criteria were met, as stated in the report conclusion, "There was no effect on print legibility, ink film integrity, print integrity, color fastness or substrate color from any of the agents tested. No print discoloration or change in solvent color was observed in this study." The photo identified in Question 19c was impacted by glare. The two additional tests from the same condition demonstrated excellent legibility, integrity and color fastness.

FDA evaluation of the Applicant's response, August 2012: Results from the ink stability study conducted by the Applicant have been reviewed and are adequate to support approval of the NDA.

20. *Regarding the Agar diffusion study-ISO, 12-603-01:*

Please confirm both the ribbon and acrylate inks were present on the label strips and came into direct contact with cells during the study.

Hemerus Response:

As stated previously, the (b)(4) ribbon ink was inadvertently missed during the original testing. Repeat testing, using all the inks, is presented in **Appendix 4** in (b)(4) Report 12-3101-G1. An Agar Diffusion Test was chosen as the appropriate test design for the solid test articles (labels). The labels were placed directly on the agar layer that protects the cells from mechanical damage while allowing the diffusion of leachable chemicals from the test article. No biological reactivity (Grade 0) was observed in the (b)(4) mammalian cells at 48 hours post-exposure to the test article. The observed cellular response obtained from the positive control

article (Grade 3) and negative control article (Grade 0) confirmed the suitability of the test system.

FDA evaluation of the Applicant's response, August 2012: Results from the Agar diffusion study are acceptable, and demonstrate that the inks used on SOLX[®] blood bag labels are not cytotoxic to mammalian cells, when immobilized to label stocks.

21. Regarding extraction procedures used on LeukoSep filters and SOLX circuits:

- a. Please confirm the LeukoSep filter was included in the SOLX circuit when the (b)(4) were performed.
- b. Please justify why an extraction of the LeukoSep filtration unit using an appropriate solvent followed by analysis of the extracts by OC/MS and HPLC/MS was not performed.
- c. Based on the total surface area of the SOLX circuit and an established extraction ratio of (b)(4) please confirm:
 - i. The extract volumes of 500 mL used in the biocompatibility studies was the appropriate amount.
 - ii. The extract volume of (b)(4) used in the metal analyses (Study 06-5803-N2) was also the appropriate amount.

Hemerus Response:

- a. Yes, the LEUKOSEP[®] filter was included in the SOLX[®] System circuit when the (b)(4) oil extractions were performed.
- b. The testing reported in BN110059 Module 4 was conducted using guidelines of ISO 10993 – *Biological Evaluation of Medical Devices* and FDA guidance document *Use of International Standard ISO-10993 Biological Evaluation of Medical Devices Part 1 Evaluation and Testing - May 1, 1995 (G95-1)*. To our knowledge, a solvent extraction followed by analysis of the extracts by GC/MS and HPLC/MS is not required and therefore was not performed.
- c.i. Biocompatibility studies were conducted using extraction principles outlined in ISO 10993-12 *Biological evaluation of medical devices – Part 12: Sample preparation and reference materials*. The extraction volume of 500 mL was determined to be appropriate based on simulated use of the system and the hazard potential related to its intended use (i.e. intended for collection and processing of 500 mL whole blood).
- c.ii. The metal analysis performed as part of Study 06-5803-N2 was conducted according to guidelines of ISO 3826 *Plastics collapsible containers for human blood and blood components – Part 1 Conventional containers Annex A.3*. A nominal volume of (b)(4) was used to perform the extraction according to Annex A.3 Preparation of the test fluid. The nominal volume was based on simulated use of the single container intended to hold approximately (b)(4) of blood product.

FDA evaluation of the Applicant's response, August 2012: The Applicant's response to part b is not acceptable. The results from a risk assessment (described in the FDA evaluation of the Applicant's responses to IR requests 16 and 17, above) should be provided, to address any safety concerns related to the filter and circuit components of the SOLX[®] system. This deficiency will be communicated to the Applicant in the CR letter.

22. Regarding (b)(4) MEM elution assay, 09-3504-01:

A (b)(4) amount of SOLX extract was applied to (b)(4) cells, but the total volume of media used during incubation of the cells was not indicated. Please provide the SOLX extract dilution factor and final SOLX extract concentrations used during these experiments.

Hemerus Response:

After consulting with the testing laboratory, it was confirmed that the total volume of media used during incubation was (b)(4). All of the maintenance media was removed (b)(4) (b)(4) prior to administration of (b)(4) of the neat SOLX[®] extract. A dilution factor was not employed in the testing.

FDA evaluation of the Applicant's response, August 2012: This response is acceptable.

23. *Regarding Chemical and physicochemical characterization of extracts, 10-1868-G1:*

Please justify why total organic carbon, OC/MS and HPLC/MS analyses were not conducted to further identify and quantify chemical components, in addition to metals and non-volatiles, in SOLX extracts.

Hemerus Response:

Testing was conducted based on USP <661> Containers, Physicochemical Test for Plastics. All results were well below the acceptance criteria and further testing was not considered necessary or required.

FDA evaluation of the Applicant's response, August 2012: The response provided is not acceptable. As stated previously, the results from a risk assessment (described in the FDA evaluation of the Applicant's responses to IR requests 16 and 17, above) should be provided to address any safety concerns related to the extractable/leachable components of the SOLX[®] system. This deficiency will be communicated to the Applicant in the CR letter.

24. Regarding the Repeat-dose toxicity study, 09-5442-G 1:

- a. Platelet counts in male mice administered SOLX extract following repeat dosing were reduced to 1075 ± 147 K/ μ L compared with 1374 ± 45 K/ μ L in saline treated male mice. Platelets were also reduced to 489 ± 348 in female mice administered SOLX extracts compared with 983 ± 306 in female mice administered saline controls. Although sample clotting was observed during this study, please still provide an explanation for decreased platelet counts and perform histopathology on splenic tissues to rule out any potential immunotoxicity.
- b. The potential of DEHP and other plasticizers present in SOLX extracts to disrupt endocrine function is a safety concern. However, results from the histological examination of rat testes tissues were not reported. If available, please provide these data.
- c. Please explain why a recovery period, to monitor the possible occurrence of delayed toxicity, was not included in the experimental design.

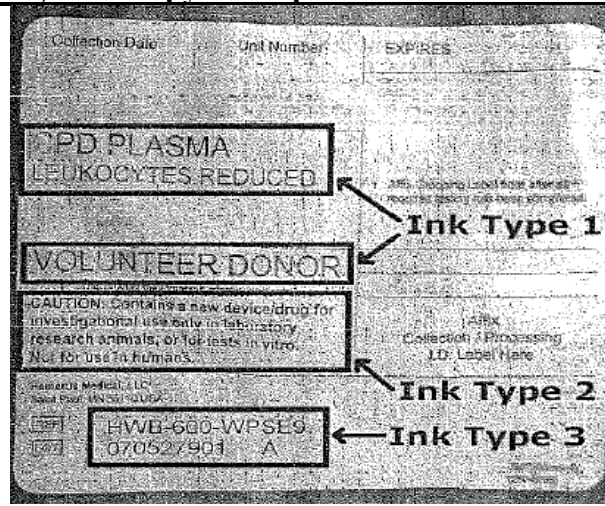
Hemerus Response:

- a. Please see the response to Question 7 which addressed the issues within Question 24.a. The testing was conducted using a recognized methodology according to ISO 10993-11, *Tests for Systemic Toxicity*. Additional testing is not planned.
- b. The data requested is not available. The safety of DEHP plasticized PVC for use in blood containers has been widely accepted and is the industry standard. As discussed in Question 9, extensive studies were performed for DEHP in Group 2 SOLX[®] RBC vs. Group 2 Control AS-1 RBC during the clinical study. At Day 42 of storage, there was no significant difference when comparing Group 2 SOLX[®] RBC to a currently licensed Group 2 Control AS-1 RBC ($p=0.79$). Therefore, the SOLX[®] System, when used according to its intended use, poses no more concern, in regards to DEHP, than the currently licensed system.
- c. As stated previously, the testing was conducted using a recognized methodology according to ISO 10993-11, *Tests for Systemic Toxicity*.

Below is the Applicant's reply to duplicate questions 7b (requested by the FDA CMC discipline) and 24a (requested by the FDA pharmacology/toxicology discipline):

7b. At the time of this study (2009), the time that blood was drawn from the animal was not recorded. Although it cannot be definitively determined, there is some possibility the entire test group was bled first and then the control group was bled; thus the platelet count would decrease over time in the test group due to spontaneous platelet aggregation. Other than this unlikely but possible explanation, the values were lower in the test males; however, they were within historical reference intervals, there were no clinical observations, and there were no histopathological findings in the selected tissues examined to indicate an effect of the test article. When excluding the values of the female animals #12 and #14 with clots in the samples (and thus lower platelets) there is no statistically significant difference between the female test and the control group (Test 728 ± 163 $n=3$ and Control 983 ± 306 $n=5$).

FDA Reviewer Comment, August 2012: Low platelet counts observed during the rat toxicity study are identified as a safety concern that has not been adequately addressed in the response submitted by the Applicant. The Applicant will be requested to address this deficiency by providing the requested risk assessment (discussed under FDA evaluation

Figure 1, Photocopy of the printed labels used on SOLX®

Results: No reactivity or clearance around cells was observed, suggesting that the inks used on these labels were not cytotoxic to mammalian cells. Additional reactivity grade results are presented in Table 1 that follows:

[(b)(4)]

II. Resistance of Hemerus bag label prints to various liquid agents, performed by Hemerus, PC410930, July 9, 2012

Purpose: To evaluate the stability of polymeric SOLX® labels printed with acrylate black and red inks manufactured by ---(b)(4)----- and ribbon ink --(b)(4)-- manufactured by (b)(4).

 -----(b)(4)-----

Figure 2, Photo of the SOLX[®] blood bag label and test strips containing 3 different inks



(b)(4)

[(b)(4)]

(b)(4)

Results: All three ink types appeared stable and maintained their integrity and legibility following exposure to all of the test agents. The results from this study, summarized below in Table 3, are satisfactory to assure ink resistance following application to the Applicant's label stock material.

1 page redacted due to (b)(4)