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US ARMY INSTITUTE OF PUBLIC HEALTH
5158 BLACKHAWK ROAD
ABERDEEN PROVING GROUND MARYLAND 21010-5403

MCHB-IP-TEP

11 March 2013

MEMORANDUM FOR Environmental Acquisition and Logistics Sustainment Program (AMSRD-FE/Ms. Kim Watts), U.S. Army Research, Development and Engineering Command, 3072 Aberdeen Boulevard, Aberdeen Proving Ground, MD 21005

SUBJECT: Toxicology Study No. S.0002733-12, Protocol No. OEYB-IV-11-11-01, *In Vitro* Dermal Absorption of Insensitive Munitions Explosive 101 (IMX-101) and Components, December 2011 – July 2012

1. An electronic copy of the final report with the Executive Summary is enclosed..
2. The U.S. Army Public Health Command, Army Institute of Public Health point of contact is Dr. Wilfred McCain. He may be contacted at DSN 584-3980, commercial 410-436-3980, or electronic mail at usaphctoxinfo@amedd.army.mil.

FOR THE DIRECTOR:

A handwritten signature in blue ink, appearing to read "Mark S. Johnson", is positioned above the printed name.

MARK S. JOHNSON
Director, Toxicology Portfolio

Encl



**Toxicology Study No. S.0002733-12, Protocol No. OEYB-IV-11-11-01,
January 2013
Toxicology Portfolio**

***In Vitro* Dermal Absorption of Insensitive Munitions Explosive 101 (IMX-101)
and Components, December 2011 – July 2012**

**Prepared by Dr. Wilfred McCain, Dr. Larry Williams, and Dr. Gunda Reddy,
Toxicity Evaluation Program**

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ABSTRACT

The U.S. Army Environmental Quality Technology program is focusing on creating new ordnance formulations for developing new insensitive munitions to replace high energetic compounds such as hexhydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 2,4,6-trinitrotoluene (TNT). There is limited information available on dermal absorption of munitions chemicals for health risk assessment. We studied *in vitro* dermal penetration of the newly developed munition IMX-101 (Insensitive Munitions Explosive-101) as a whole composition and its individual components of 3-nitro-1,2,4-triazol-5-one (NTO), 2,4-dinitroanisole (DNAN), and nitroguanidine (NQ) in static Franz diffusion cells. In this system, the human epidermal membranes were prepared from frozen cadaver skin and were mounted on static diffusion receptor cells so that the visceral side was intact with the receptor fluid. Test chemicals at infinite dose (100 milligrams (mg)) powder were carefully placed on the mounted skin in the donor chamber, and at different times (1, 2, 4, 6 and 8 hours) 0.1 milliliter (mL) of receptor fluid (buffer) was collected and quantified by High Performance Liquid Chromatography. The effect of personal protective equipment (Tyvek[®]) was also tested by placing the applied dose of IMX-101 (10 mg) on the cells. The penetration rate was calculated in micrograms per square meter per hour ($\mu\text{g}/\text{cm}^2/\text{hr}$) for each chemical. The analysis of absorbed chemical in the receptor fluid data showed that steady state of fluxes of infinite dose of neat NTO, DNAN, and NQ were 338.2, 1.1, and 31.3 $\mu\text{g}/\text{cm}^2/\text{hr}$, respectively. The IMX-101 powder (100 mg) had a rate of steady flux for DNAN, NTO, and NQ were 1.8, 135.9, and 236.3 $\mu\text{g}/\text{cm}^2/\text{hr}$, respectively. NTO and NQ showed about 0.4 and 7 times more rate of penetration in the combination of explosive. The penetration of IMX101 with Tyvek showed the penetration flux of NTO, DNAN, and NQ were 102.5, 1.9, and 46.4 $\mu\text{g}/\text{cm}^2/\text{hr}$, respectively. These estimated *in vitro* human model values will be used to evaluate health risk associated with dermal exposure. (Tyvek[®] is a registered trademark of E.I. du Pont de Nemours and Company.)

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Study Title

In Vitro Dermal Absorption of Insensitive Munitions Explosive 101 (IMX-101) and Components

Data Requirement

OECD 428 Guideline for Testing of Chemicals

Authors

Wilfred McCain, Ph.D.
Larry William, Ph.D.
Gunda Reddy, Ph.D., D.A.B.T.

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17 July 2012

Performing Laboratory

U.S. Army Public Health Command
Army Institute of Public Health
Toxicology Portfolio
Toxicity Evaluation Program (MCHB-IP-TEP)
5158 Blackhawk Road
Aberdeen Proving Ground, MD 21010-5403

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study described in this report was conducted in compliance with Title 40, Code of Federal Regulations (CFR), Part 792, Good Laboratory Practice Standards, except for the following:

No deviations from the aforementioned regulation affected the quality or integrity of the study or the interpretation of the results.



Wilfred McCain, PhD
Study Director

20130227

Date



Arthur O'Neil
Program Manager
Toxicity Evaluation

20130227

Date

Toxicology Study No. S.0002733-12, Protocol No. OEYB-IV-11-11-01
***In Vitro* Dermal Absorption of Insensitive Munitions Explosive 101 (IMX-101) and**
Components
December 2011 – July 2012

1 Summary

1.1 Purpose

To determine the ability of Insensitive Munitions Explosive 101 (IMX-101) and its individual components 3-Nitro-1,2,4-triazol-5-one (NTO), 2, 4-Dinitroanisole (DNAN), and Nitroguanidine (NQ) to penetrate skin and to correlate this information with *in vivo* results.

1.2 Conclusions

The analysis of the absorbed chemical in the receptor fluid data showed that steady state fluxes to an infinite dose (100 milligrams (mg)) of neat NTO, DNAN, and NQ were 338.2, 1.1, and 31.2 micrograms per square meter per hour ($\mu\text{g}/\text{cm}^2/\text{hr}$), respectively. While when whole IMX-101 tested at 100 mg powder, the rates of steady fluxes showed for NTO, DNAN, and NQ were 135.9, 1.8, and 236.3 $\mu\text{g}/\text{cm}^2/\text{hr}$, respectively. NTO and NQ showed about 0.4 and 7 times more rates of penetration from the combination of IMX-101 explosive. The penetration of IMX-101 at a dose of 10 mg with Tyvek covered on it showed the penetration fluxes of NTO, DNAN, and NQ were 102.5, 1.9, and 46.4, $\mu\text{g}/\text{cm}^2/\text{hr}$ respectively.

1.3 Recommendations

Use the measured values from the *in vitro* human epidermal model to evaluate health risk associated with dermal exposure.

2 References

Appendix A contains a list of references used to prepare this report.

3 Background

The U.S. Army Environmental Quality Technology (EQT) program is focusing on creating new ordnance formulations in developing new insensitive munitions to replace high energetic compounds such as hexhydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 2,4,6-trinitrotoluene (TNT). Dermal exposure to these ammunition chemicals is a major concern for the Army. Skin is a potential route for absorption of chemical components of explosives. The dermal absorption of munitions chemicals may occur during manufacture, loading, assembly, and use as well as during field operations. The information on skin absorption of munition chemicals is limited. Nitroaromatic compounds are reported to readily penetrate the skin and produce toxic effects. Occupational exposure to munitions containing TNT and 1,3-dinitrobenzene (DNB) has shown that these munitions are able to penetrate skin and cause methemoglobinemia in human volunteers (Ishihara et al. 1976; Woolen et al. 1986). The percutaneous absorption of five nitroaromatic compounds through excised human skin showed that a maximum rate of absorption occurred within 2 hours after application of the compound to the skin (Bronaugh and Maibach 1985).

Kraeling et al. (1998) studied percutaneous absorption of 1,3,5-trinitrobenzene (TNB) in acetone or water using the viable skin excised from hairless guinea pigs, rats, and human skin from cosmetic surgery. These studies showed that TNB was significantly lower with human skin when compared to that of rats and guinea pigs. Reifenrath et al. (2002) studied the percutaneous absorption of explosives and related compounds *in vitro* using pig skin and showed that skin absorption of nitro compounds from soil was found to correlate with water solubility and vapor pressure. They showed that TNT penetration in all vehicles (acetone or soils) was highest in the first 2 hours of dosing. Reddy et al. (2008) studied percutaneous absorption of RDX in excised human skin *in vitro* and showed that approximately 5.7% of the RDX was found in the skin (epidermis, dermis, and receptor fluid) over a 24-hour period when applied in acetone.

Information on the dermal absorption of IMX-101 or its individual components is limited.

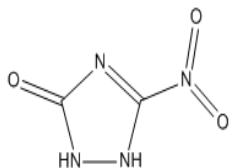
4 Materials and Methods

4.1 Chemicals

The test articles NTO, 99.6 % pure, CAS # 932-64-9, Lot # BAE 07B 305001 and neat DNAN, CAS # 119-27-7, 100% pure, Lot # BAE 10H281-008 were obtained from BAE Systems, Ordnance Systems, Inc., Kingsport, Tennessee. These chemicals were gently ground using a mortar and pestle to a fine consistency before weighing the required amount for testing. The test article NQ, CAS # 556-88-7, 99.90% pure in 25% of water was obtained from Sigma Aldrich. The water content was removed and dried in the hood and grounded gently before use. The test material IMX-101, Lot # BAE 10H375-046 was synthesized and provided by the BAE Systems, Ordnance Systems, Inc. The IMX-101 was ground dry in a mortar and pestle to a fine consistency and sifted through a 20-mesh screen. The compound purity was performed by the manufacturer and reported as 99.6% pure by High Performance Liquid Chromatography (HPLC). The purity of these chemicals was further confirmed by the Army Institute of Public Health Laboratory Services (AIPH LAB) and found to have similar values.

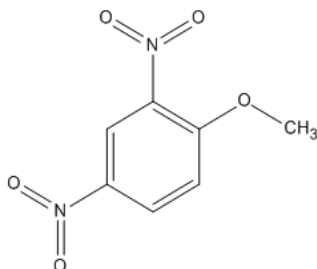
Tyvek material was obtained by cutting circular pieces with an 18-millimeter (mm) metallic Arch Punch (Osborne and Company) from a Tyvek Sleeve Protector (DuPont Personal Protection, Lot # 03065340).

4.1.1 3-Nitro-1,2,4-triazol-5-one



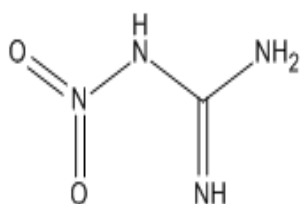
NTO is an energetic explosive, presently used in a number of formulations in weapon systems. No *in vivo* or *in vitro* dermal absorption studies are reported and no occupational dermal exposure data is available (Appendix C).

4.1.2 2,4-Dinitroanisole



DNAN was historically used as an explosive in warheads containing Amatol and is currently being investigated as replacement for TNT in melt cast insensitive munitions (IM) formulations. There is no *in vivo* dermal absorption data available in animals or humans. Only one *in vitro* dermal penetration study was found in the literature. DNAN is one component of the explosive composition, PAX-21 (~ 34%). The *in vitro* dermal absorption of DNAN was studied in excised rat skin *in vitro*. When a 0.5 gram of PAX-21 was applied to the excised rat skin and dermal penetration in receptor fluid was evaluated for 6 hours. During first 2 hours there was no difference in base line levels. The steady state flux from 2 to 6 hours was 0.74 $\mu\text{g}/\text{cm}^2/\text{hr}$ (McDougal et al. 2000). The dermal penetration of pure DNAN was also studied to compare the dermal absorption of DNAN in PAX-21. DNAN penetrated the skin when applied in pure powder on the excised rat skin in static diffusion cell. The flux of pure DNAN for the period from 2 to 6 hours was 1.55 $\mu\text{g}/\text{cm}^2/\text{hr}$. This is approximately two-fold higher than the flux of DNAN from PAX-21 (CBR-12).

4.1.3 Nitroguanidine



NQ is a nitroamino compound. It is a colorless crystalline solid. It was used in military munitions during World Wars I and II. No studies on the health effects of NQ in humans have been reported. No data on dermal absorption of NQ were located in occupational exposure studies or *in vivo* animal studies. The *in vitro* dermal absorption of M30A1 propellant powder (500 mg; nitroguanidine, 47.7%; nitrocellulose, 28%; nitroglycerine, 22.5%) used in the modular artillery charge (XM232) was studied in the excised skin of rats. Only nitroglycerine penetrated 0.03 $\text{mg}/\text{cm}^2/\text{hr}$ but no flux values could be determined with other chemicals (McDougal et al. 1998). This data show that NQ is not readily absorbed through the skin of rats. The dermal permeability coefficient (K_p) of 1.03^{-4} for NQ was predicted on the partition coefficient ($K_{o/w}$) of -0.89 was very low. This is about 11 times less than nitroglycerine which has a K_p value of 1.11^{-3} (McDougal and Jepson 1998).

4.1.4 Insensitive Munition Explosive

IMX-101 is a composition of NTO, DNAN, and NQ. It is a new ordnance formulation developed to replace high energetic compounds. There is no information on its dermal absorption in animals and occupational exposure.

Therefore, we studied the dermal absorption of the individual components of IMX-101 as well as the compounded formulation using human skin in a static Franz cell diffusion system.

4.2 Human Skin Preparation

Full thickness abdominal human skin (from autopsy or surgery) was obtained from the National Disease Research Interchange (NDRI), Philadelphia, Pennsylvania. The whole skin was stored frozen at -85 degrees Centigrade (°C). Before starting the experiments, the skin was placed in a Petri dish containing a buffer solution at room temperature. The subcutaneous fat was removed with blunt forceps, and the skin was cleaned with a buffer solution and placed on a dissecting board. Circular pieces of skin sections (0.64 cm²) were prepared from the skin with the help of a metallic Arch Punch (18 mm; Osborne and Company). The epidermis was teased from dermis as described previously (Frasch et al. 2011). The skin discs were wrapped in Saran® Wrap and submerged in a 60°C buffer for approximately 70 seconds. The skin was unwrapped and placed in a Petri dish containing a buffered solution. The dermis of the skin discs was teased from the epidermis carefully using blunt forceps or cotton swabs under a binocular microscope. Care was taken not to damage the skin. During epidermal membrane separation, a thin layer of stratum corneum was easily separated from the skin. The epidermal membranes remaining were used for testing. The epidermal membranes were evaluated under a dissection microscope to ensure that they were free of any damage. The epidermal membranes (without stratum corneum) were placed on wax paper moistened with a buffered solution and frozen (-30°C) prior to experimental use. Before use, the stored epidermal membrane discs were thawed at room temperature and checked again for any damage under the dissection microscope before testing. The use of heat-separated human skin epidermal membrane is in accordance with the guidelines of the Organization for Economic Cooperation and Development for the conduct of *in vitro* dermal penetration studies (OECD 2004) (OECD 428). (Saran® Wrap is a registered trademark of the Dow Chemical Company.)

4.3 Franz Diffusion Cell

The Franz diffusion cell is one of the most widely used systems for *in vitro* skin permeation studies. The *in vitro* static diffusion cell system (PermeGear, Inc., Hellertown, Pennsylvania) was composed of water-jacketed glass cells with a receptor chamber volume of 5 milliliters (mL). The individual cells were coupled to a water distribution system, which was connected to a recirculating water bath adjusted to allow the skin, mounted on the *in vitro* cells, to be maintained at 32°C. Single Franz diffusion cells were mounted in a rack which can hold six cells. The exposed skin surface was 0.64 cm². The receptor chamber was filled with 0.9% phosphate buffered saline (PBS) and the skin disk was placed above. The donor chamber was clamped over the skin disk on the receptor chamber. The donor chamber is equipped with a side arm which allows for the collection of samples from the receptor fluid for the measurement of penetrated chemicals. The system was allowed to equilibrate before measuring skin integrity.

4.4 Electrical Resistance

The human skin may be damaged during surgery or during the separation of epidermal membranes. Several methods are described to test skin integrity. One method, developed by Bronaugh et al. 1986, evaluates the tritiated ($^3\text{H}_2\text{O}$) water permeability coefficient to test skin integrity. Fasano et al. (2002) tested *in vitro* dermal integrity by measuring electrical impedance and comparing these values with that of tritiated water and proposed acceptable values to evaluate epidermal membranes. A Tinsley LCR Databridge (Model 6451, Redhill, Surrey, United Kingdom) was used to measure electrical impedance. PBS (5 mL) was placed in the receiver chamber, skin (epidermal side up) was placed on top of the ground glass joint, and the donor chamber was clamped in place. Approximately 1 mL of PBS was added to the donor chamber. The system was allowed to equilibrate for 15 minutes. Copper electrodes were placed in the donor solution and receiver fluid (PBS) and impedance were measured both before and after the experiment. Impedance measurements were taken at (a) Parallel-equivalent (PAR), 100 Hz; (b) Series-equivalent (SER) 100 Hz; (c) PAR, 1000 Hz; and (d) SER, 1000 Hz. Impedance values are reported in kilo-ohms (k Ω). The buffer fluid from the donor chamber was carefully removed with a pipette and gently dabbed with cotton swabs to remove any solution on the skin, and the test substance was added to the surface of the moistened skin in the donor chamber. Regression analyses of impedance data were performed using Microsoft Excel[®] 2007. (Microsoft Excel[®] is a registered trademark of the Microsoft Corporation.)

4.5 Sample Testing

Individual chemical samples of powdered material were applied to the moistened human epidermal membranes in the donor chamber at an infinite dose (100 mg). This dose was selected on the basis of previously tested munitions compounds such as DNAN and NQ by McDougal et al. (2000 and 1998), where 500 mg of dry powder *in vitro* rat skin epidermal membrane penetration. At different times, 0.1 mL of receptor fluid was collected with a syringe and was placed in a vial containing acetonitrile (0.9 mL) for further dilution and analysis by HPLC by the AIPH LAB Portfolio. The volume of the receptor fluid was maintained at 5 mL by replenishing it with 0.1 mL of buffer after each sample collection. Samples were collected at 1, 2, 4, 6, 8, and 24 hours for NTO. The steady state flux of NTO was between 6 to 8 hours. Therefore, the other chemicals were tested for 8 hours to determine steady state flux. The steady state flux of each chemical tested was calculated to determine the chemical's rate of penetration. Personal protective equipment (PPE, Tyvek) was also tested by preparing 0.64 cm² circular pieces of PPE, which were placed on an applied IMX-101 chemical dose of 10 mg in the donor chamber. The receptor fluid was collected as described earlier.

4.6 Quality Assurance

The U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) Good Laboratory Practice Policy—Policy Memorandum 74 states — All experiments and studies conducted by any element of the AIPH Portfolio of Toxicology (formerly USACHPPM) will be compliant with the applicable Good Laboratory Practice (GLP) Standard guideline (USACHPPM 2009). For this study, the test article dictates that the following regulation applies:

Title 40, Code of Federal Regulations (CFR), Part 792, Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards: Final Rule

According to this policy and that these results may be used in regulatory decisions involving the EPA, this study was conducted in compliance with TSCA GLP standards and followed the appropriate regulatory testing guidelines. In compliance with the GLP requirements, the AIPH Quality Systems Office audited critical phases of this study. A Quality Assurance Statement is provided in Appendix B, which provides the dates of these audits along with the audited phases and the dates that the results of the audits were reported to Management and the Study Director. The additional GLP requirement of archives location is provided at the end of Appendix C and E as well as the names of personnel contributing to the performance of this study.

5 Findings and Discussion

The skin electrical impedance (EI) described above was measured for each test before starting and completing experiments. The results show that all cell EI have consistent values at different levels of PAR and SER before and after termination. There is a slight decrease in impedance values at 8 hours when compared to the start of the experiments. The EI values of NTO were at 24 hours (Table 1). The regression analysis of impedance data show very close acceptable values.

The percutaneous absorption of the individual chemicals (NTO, DNAN and NQ) and the IMX-101 whole composition powder (mixed) in human skin in the *in vitro* system was studied, and the results are presented in the tables and figures below. The details of EI raw data, chemical compounds, and tests with PPE are presented in Appendix D.

First studied was the NTO penetration at different times up to 24 hours to determine steady state flux. The results of NTO presented in Figure 1 show that steady state of absorption was found from 6 to 8 hours, and the rate of penetration did not change afterwards. Therefore, we studied all penetration studies of other chemicals up to 8 hours. The study state flux at 8 hours was 338.2 $\mu\text{g}/\text{cm}^2/\text{hr}$. This value is the average penetration for values at different hours of collected samples (1, 2, 4, 6, and 8 hours) (Table 2). NTO as a component of IMX 101 absorbed at the rate of 135.9 $\mu\text{g}/\text{cm}^2/\text{hr}$ at 8 hours. This is about 2.4 times less when compared to the individual compounds applied. This may be due to the amount of dose (100 mg) when applied as an individual chemical or it may be due to the chemical composition where NTO is only 19% of total 100 mg of IMX-101 applied. The cumulative dermal absorption of NTO was found to be 5843 $\mu\text{g}/\text{cm}^2$, while at 24 hours the cumulative absorption was 4250 $\mu\text{g}/\text{cm}^2$ (Table 4).

The dermal absorption rate for DNAN at 8 hours was 1.1 $\mu\text{g}/\text{cm}^2/\text{hr}$ when tested separately, while in the mixture of IMX-101 the rate was 1.8 $\mu\text{g}/\text{cm}^2/\text{hr}$ (Table 2). The absorption rate is a very similar amount when tested individually and as a component of IMX-101. The cumulative dose in 8 hours was 12.5 $\mu\text{g}/\text{cm}^2$ (Table 4).

The dermal absorption rate for NQ was about 31.2 $\mu\text{g}/\text{cm}^2/\text{hr}$ when tested as an individual compound, compared to 236.3 $\mu\text{g}/\text{cm}^2/\text{hr}$ in the mixture of IMX-101 (Table 2). This is about seven times higher than when compared to the individual rate of penetration. The cumulative dermal absorption for NQ was 351.6 $\mu\text{g}/\text{cm}^2$ in 8 hours (Table 4).

The results of dermal penetration of IMX-101 composition (10 mg) when applied to skin covered with PPE (Tyvek) showed the rates of penetration of NTO, DNAN and NQ at 102.5, 1.9, and 46.4 $\mu\text{g}/\text{cm}^2/\text{hr}$, respectively (Table 3). The cumulative absorption in 8 hours was 416.7 $\mu\text{g}/\text{cm}^2$ for NTO, 16.8 $\mu\text{g}/\text{cm}^2$ for DNAN, and 304.7 $\mu\text{g}/\text{cm}^2$ for NQ (Table 4).

Table 1. Average impedance values for IMX-101 and individual components of NTO, DNAN, and NQ taken immediately prior to and immediately following the study period

Average impedance values (k Ω)								
	S-100		S-1000		P-100		P-1000	
	Start	8-hour	Start	8-hour	Start	8-hour	Start	8-hour
NTO *	1.68 ± 0.7	1.33 ± 0.08	2.44 ± 0.30	1.30 ± 0.8	1.32 ± 0.08	1.32 ± 0.08	1.49 ± 0.1	1.29 ± 0.07
DNAN	1.66 ± 0.08	1.68 ± 0.08	1.58 ± 0.07	1.58 ± 0.07	1.67 ± 0.08	1.68 ± 0.07	1.59 ± 0.08	1.59 ± 0.07
NQ	1.75 ± 0.05	1.46 ± 0.06	1.55 ± 0.07	1.37 ± 0.06	1.78 ± 0.12	1.45 ± 0.06	1.54 ± 0.07	1.37 ± 0.06
IMX-101	1.54 ± 0.09	1.37 ± 0.05	1.33 ± 0.7	1.31 ± 0.05	1.6 ± 0.10	1.36 ± 0.07	1.35 ± 0.07	1.30 ± 0.05

Notes:

*NTO experiments measured at 24 hours. Average values of six replicates, $\pm$ SE

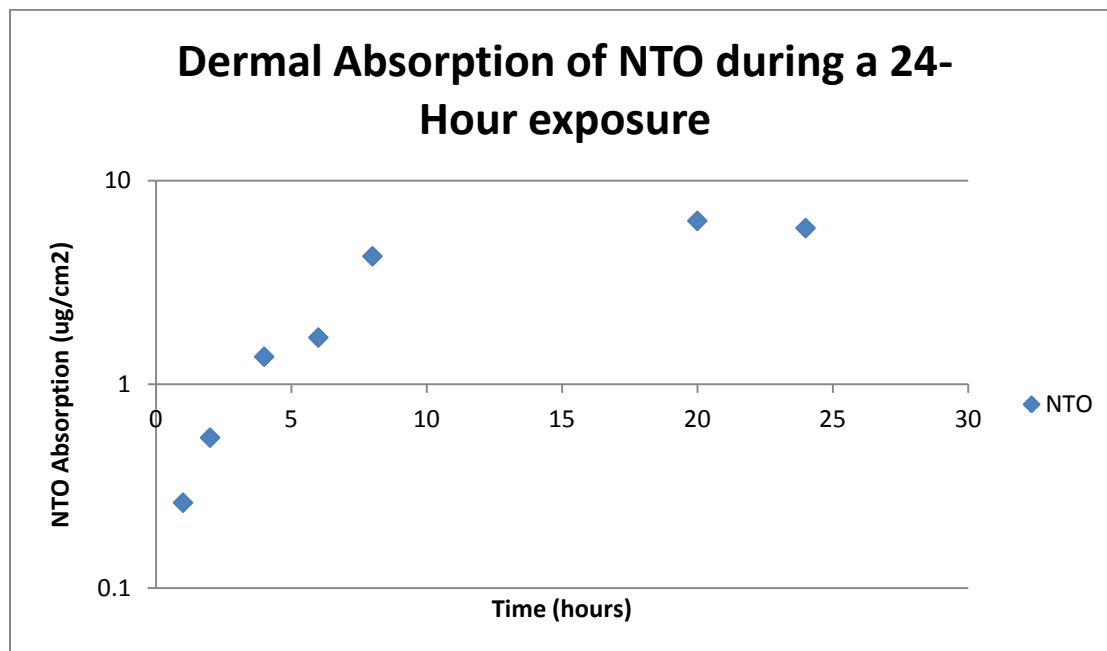


Figure 1. Dermal penetration of NTO in human skin

Table 2. Dermal absorption rate for individual and compounded components of IMX-101 at infinite dose (100 mg) following 8 hours of exposure to human epidermal membranes

Absorption rate ($\mu\text{g}/\text{cm}^2/\text{hr}$)*		
Component	Individual compounds	IMX-101
NTO	338.2 ± 238.2	135.9 ± 55.5
DNAN	1.1 ± 0.6	1.8 ± 1.0
NQ	31.2 ± 17.9	236.3 ± 47.6

*Average rates of absorption at different times during 8 hrs, values of 6 replicates

Table 3. Dermal absorption rate for individual components of IMX-101 at dose (10 mg) following 8 hours of exposure to human epidermal membranes with Tyvek PPE

Component	IMX-101 explosive ($\mu\text{g}/\text{cm}^2/\text{hr}$)*
NTO	102.5 ± 21.3
DNAN	1.9 ± 0.9
NQ	46.4 ± 15.5

* Average rates of absorption at different times during 8 hrs, values of 6 replicates

Table 4. Cumulative dermal absorption of munition chemicals following 8 hours of exposure in human epidermal membranes

Component	Individual components (100 mg)	IMX-101 (100 mg)	IMX-101 with Tyvek (10 mg)
	$\mu\text{g}/\text{cm}^2$		
NTO	4250 ± 1905	989.6 ± 443.8	416.7 ± 170.1
DNAN	12.5 ± 5.1	17.4 ± 7.8	16.8 ± 6.9
NQ	351.6 ± 143.5	849.0 ± 380.7	304.7 ± 124.4

6 Conclusions

The analysis of absorbed chemical in the receptor fluid data showed that steady state fluxes to an infinite dose (100 mg) of neat NTO, DNAN, and NQ were 338.2, 1.1, and 31.3 $\mu\text{g}/\text{cm}^2/\text{hr}$, respectively, *in vitro* human skin epidermal membrane. When tested IMX-101 (100 mg) powder, the rate of steady fluxes showed for NTO, DNAN, and NQ were 135.9, 1.8, and 236.3 $\mu\text{g}/\text{cm}^2/\text{hr}$, respectively. NTO and NQ showed about 0.4 and 7 times more rate of penetration in the combination explosive IMX mixture. The penetration of IMX-101 (10mg) with Tyvek placed on the chemical showed the penetration fluxes of NTO, DNAN, and NQ were 102.5, 1.9, and 46.4 $\mu\text{g}/\text{cm}^2/\text{hr}$, respectively.

7 Recommendation

Use the measured values from the *in vitro* human epidermal model to evaluate health risk associated with dermal exposure.



WILFRED C. McCAIN
Toxicologist
Toxicity Evaluation Program

Approved:



MARK S. JOHNSON
Director, Toxicology Portfolio

APPENDIX A

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APPENDIX B
QUALITY ASSURANCE STATEMENT

QUALITY ASSURANCE STATEMENT

For: Toxicology Protocol No. OEYB-IV-11-11-01, entitled: " *In Vitro* Dermal Absorption of Insensitive Munitions Explosive 101 (IMX-101) and Components" the following critical phases were audited by the USAPHC Quality Systems Office:

PRE IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Study Protocol GLP Review	10/24/2011	10/24/2012

IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Test Article – NTO Control Procedures	12/22/2011	12/29/2012
Test Article – NTO – Handling Procedures	12/22/2011	12/29/2012
Test Article – NTO – Administration Procedures	12/22/2011	12/29/2012
Reference Substances - Verification of Appropriate Reference Substances	12/22/2011	12/29/2012
Test System - Skin Preparation Integrity Confirmation	02/03/2012	02/14/2012
Compliance with TOX Protocol Approval Requirements	02/03/2012	02/14/2012
Compliance with TOX SOP Requirements	02/03/2012	02/14/2012
Analytical Chemistry Support – DNAN - Dermal Absorption Determination Analysis by LAB	03/15/2012	03/21/2012

POST IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Final Study Report Review	12/14/2012	12/18/2012
Study Raw Data Review	12/14/2012	12/18/2012

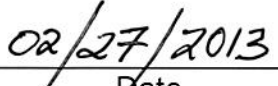
Note 1: All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.

Note 2: In addition to the study specific critical phase inspections listed here, general facility and process based inspection not specifically related to this study are done monthly or annually in accordance with QA Standard Operating Procedures.

Note 3: This report has been audited by the Quality Assurance Unit (QSO), and is considered to be an accurate account of the data generated and of the procedures followed.



Michael P. Kefauver
GLP Assessor, QSO



Date

APPENDIX C
STUDY PROTOCOL

**IN VITRO PROTOCOL
TOXICOLOGY DIRECTORATE
U.S. ARMY PUBLIC HEALTH COMMAND
ABERDEEN PROVING GROUND, MD 21010-5403**

PROTOCOL TITLE:

In vitro Dermal Absorption of Insensitive Munitions Explosive 101 (IMX-101) and components.

PROTOCOL NUMBER:

OEYB-90-iv-11-11-01

PRINCIPAL INVESTIGATOR/STUDY DIRECTOR:

Wilfred C. McCain, Ph.D.
Senior Toxicologist
Toxicity Evaluation Program
Toxicology Directorate (DSN 584-2201)
U.S. Army Public Health Command
Aberdeen Proving Ground, MD 21010-5403

CO-INVESTIGATOR(S):

Gunda Reddy, Ph.D., DABT
Senior Toxicologist
Health Effects Research Program
Toxicology Portfolio (DSN 584-7701)
U.S. Army Public Health Command
Aberdeen Proving Ground, MD 21010-5403

Larry Williams, Ph.D.
Biologist
Health Effects Research Program
Toxicology Portfolio (DSN 584-7159)
U.S. Army Public Health Command
Aberdeen Proving Ground, MD 21010-5403

TECHNICAL ASSISTANTS:

Terry Hanna
Senior Technician
Toxicity Evaluation Program
Toxicology Portfolio (DSN 584-5072)
U.S. Army Public Health Command
Aberdeen Proving Ground, MD 21010-5403

Mark Way
Biologist
Toxicity Evaluation Program
Toxicology Portfolio (DSN 584-5089)
U.S. Army Public Health Command
Aberdeen Proving Ground, MD 21010-5403

SPONSOR:

I. NON-TECHNICAL SYNOPSIS:

The test substance, in this case a solid, is applied to the surface of a skin sample separating the two chambers of a diffusion cell. A diffusion cell consists of a donor chamber and a receptor chamber between which the skin is positioned. The material remains in contact with the skin for a specified time (4 hours) under specified conditions. The receptor fluid is sampled at time points (30, 60, 120 and 240 minutes) throughout the experiment and analyzed for the test chemical.

II. BACKGROUND:

II.1. A number of papers have been published describing *in vitro* testing for skin absorption (references 1-24). This type of testing has several advantages over *in vivo* methods. For instance, human skin as well as that of other animals can be used, replicate measurements can be obtained, live animals are not used, intended use exposure conditions can be studied, a wider range of physical forms can be investigated including solids and granules, and the impact of skin damage on absorption can be assessed avoiding ethical issues. The *in vitro* method is rapid and inexpensive.

The Organization for Economic Co-operation and Development (OECD) has developed a guidance document for the conduct of skin adsorption studies (reference 13) and the U.S. Environmental Protection Agency has proposed a similar *in vitro* test method (reference 9). Also, the EPA requires under the Toxic Substances Control Act (TSCA) that manufacturers (including importers) and processors of 34 chemicals conduct *in vitro* dermal absorption rate testing. "These chemicals are of interest to the

Occupational Safety and Health Administration (OSHA) of the Department of Labor, and the data obtained under this testing program will be used by OSHA to evaluate the need for “skin designations” for these chemicals. Skin designations are used by OSHA to alert industrial hygienists, employers, and workers to the potentially significant contribution to the overall exposure to certain chemicals which can occur by the cutaneous route.” (reference 10)

II.2. OBJECTIVE/HYPOTHESIS:

To determine the ability of IMX-101 and its individual components [3-Nitro-1,2,4-triazol-5-one (NTO), 2, 4- Dinitroanisoie (DNAN), Trinitrotoluene (TNT) and Nitroguanidine] to penetrate skin and to correlate this information with *in vivo* results.

III. MILITARY RELEVANCE:

IMX-101 is a new high explosive designed by the U.S. Army and BAE Systems, expected to be first fielded in 2011. It is intended for use in explosive shells and other munitions. 'IMX' stands for *Insensitive Munitions eXplosive*.

IMX-101 is intended to replace the current TNT and Composition B used by the U.S. in explosive artillery shells. The reason for the replacement is that IMX-101 is even more stable than TNT, which when invented was hailed as famously insensitive to shock and other conditions which would reliably detonate other high explosives of the day such as guncotton and nitroglycerine. The new explosive will reduce accidental detonation from poor handling or other shock in storage, and will even lower the likelihood that munitions will be detonated by nearby explosion. In tests, shells filled with IMX-101 did not detonate even when struck by the shaped charge of a RPG. This means that transporting and storing munitions filled with this compound will be safer in combat zones.

One advantage of IMX-101 is that it is compatible with current fuzing systems. At present, it has been certified for use in 155mm artillery shells, and is in testing in smaller 105mm shells. Another compound, IMX-104, has been announced (but not tested) for use in smaller munitions.

IMX-101 was developed by the Army and BAE at the Army's Holston Army Ammunition Plant in Kingsport, TN.

IV. MATERIALS AND METHODS:

IV.1. Experimental Design and General Procedures:

IV.1.1: Test Substance(s):

IMX-101 and its individual components [3-Nitro-1,2,4-triazol-5-one (NTO), 2,4-Dinitroanisoie (DNAN), and Nitroguanidine (NG)].

IV.1.2: Skin

Human skin will be obtained from the National Human Tissue Resource Center of the National Disease Research Interchange¹. The cadaver skin, from the abdominal region or thigh, will be split thickness skin (200-400 µm thick) prepared with a dermatome (Padgett, Kansas City, MO) or heat separated. Heat separation requires that cadaver skin be submersed in 60°C (140°F) buffer for 45 seconds. The epidermis teased from the dermis using cotton swabs. Epidermal membranes will be stored frozen (-85°C on gauze pads saturated with buffer plus 10 percent glycerol) prior to use.

Living skin equivalent models will also be employed to assess percutaneous absorption. They consist of reconstituted epidermis, grown in tissue culture and used as alternatives to animal tissues (e.g. MatTek, Ashland, MA) (reference 14, 22). EpiDerm™ is a normal (non-transformed), human cell-derived, metabolically active, 3-dimensional organotypic in vitro skin model. It closely mimics human epidermis, both structurally and biochemically, and does so in a very reproducible manner. For this series of tests, MatTek's EPI-606, (normal, human-derived epidermal keratinocytes (NHEK) which have been cultured to form a multilayered, highly differentiated model of the human epidermis, 22 mm diameter) will be used.

IV.1.3: Diffusion cell

The Franz diffusion cell is one of the most widely used systems for in vitro skin permeation studies (reference 12). Franz-type diffusion cell systems are relatively simple in design; the receptor fluid beneath the skin is manually sampled by removing aliquots periodically for analysis (reference 4). With this type of apparatus, any type or any amount of vehicle may be applied to the skin. Franz cells used for this series of tests will be 9mm jacketed cells with a flat ground (ground o-ring) joints and clear glass with a 5ml receptor volume (PermaGear, Hellertown, PA). This is the most common variety of Franz Cell made.

IV.1.4: Receptor fluid

An important factor that has to be considered, especially in static diffusion systems, is the solubility of the test compound in the receptor fluid. This may affect the sink capacity and would have an influence on the receptor chamber dimensions or sampling frequency (reference 2). For this series, Hanks Balanced Salt Solution (HBSS) or (Roswell Park Memorial Institute) RPMI 1640 (Sigma-Aldrich, St. Louis, MO) will be used as receptor fluids. When testing hydrophobic chemicals, polyethoxyoleate (polyethylene glycol (PEG) 20 oleyl ether) will be added to the receptor fluid at a concentration of 6% to facilitate sink conditions for the chemical being tested. The general guidance on how the chemicals will be applied to the skin: (1) if the chemical is a liquid at room temperature it will be applied neat; (2) if the chemical is a solid, or if it damages the skin when applied neat (namely, the stratum corneum), it will be applied in a water vehicle; (3) if the concentration of the chemical in the water vehicle was determined not to be sufficient to achieve the experimental endpoints, the chemical will

be applied in isopropyl myristate (IPM) vehicle (since it's likely that for a highly lipophilic chemical solubility in IPM would be maximized). The precise composition of the receptor fluid used for each compound in this series will be provided.

IV.1.5: Transcutaneous Electrical Resistance (TER).

Electrical impedance will be used to confirm skin integrity for *in vitro* dermal regulatory testing and as a tool to evaluate skin condition as well as to determine the irritation and corrosion potential of the various chemicals. Following equilibration with 0.9% phosphate buffered saline or RPMI 1640 in the donor and receptor chambers, an impedance measurement will be taken with a Tinsley LCR Databridge Model 6401 set in the resistance mode (R) and in (a) the serial-equivalent mode (SER) with an alternating current (AC) frequency of 100 hertz (Hz), (b) SER and 1000 Hz, (c) parallel-equivalent mode (PAR) and 100 Hz, and (d) PAR and 1000 Hz. (reference 11).

IV.1.6: Method:

Guidance for conduct of the *in vitro* dermal kinetic experiments was posted in the United States FR, April 26, 2004 (Volume 69, Number 80), pages 22402–22441, “*In Vitro* Dermal Absorption Rate Testing of Certain Chemicals of Interest to the Occupational Safety and Health Administration” (). The tests will be carried out in accordance with “OECD Guideline for the Testing of Chemicals: Guideline 428: Skin Absorption: *in vitro* method” (OECD, 2004, Appendix 2) and the Draft OECD Guidance Document for the Conduct of Skin Absorption Studies (OECD, 2004c).

Testing will be conducted on both human and/or artificial (reconstructed) skin using jacketed Franz cells maintained at 32 ± 1 °C. Humidity should be between 30 and 70 percent. An exposure duration of four hours was selected with 4 ml samples taken at 1, 30, 60, 120 and 240 minutes. Samples will be processed by the Directorate of Laboratory Sciences and the rate of dermal absorption for each chemical will be determined.

In addition, USAPHC Good Laboratory Practice (GLP) Policy - Policy Memorandum 74 states: “All experiments and studies conducted by any element of the USAPHC Toxicology Portfolio will be compliant with the applicable Good Laboratory Practice (GLP) guidelines reflected in the following regulations:

- a. Title 21 Code of Federal Regulations (CFR) Part 58, Good Laboratory Practice Regulations for Nonclinical Laboratory Studies.
- b. Title 40 Code of Federal Regulations (CFR) Part 160, Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Good Laboratory Practice Standards: Final Rule.
- c. Title 40 Code of Federal Regulations (CFR) Part 792, Toxic Articles Control Act (TSCA), Good Laboratory Practice Standards: Final Rule.”

This policy along with the fact that the results of these assays may be used in regulatory decisions and may be submitted to the EPA or FDA, these *in vitro* dermal

tests will be conducted in compliance with Good Laboratory Practice standards and will follow the appropriate regulatory testing guidelines depending on the test article. Each time a new test article is tested, the test system will be drawn against this protocol, as needed, by completion and approval of the In-Vitro Amendment/Modification Form (see Appendix D of DTOX SOP 85), which will identify the test article, background, military relevance, a literature review of the test article for duplication, a unique protocol number, and other pertinent information along with the signatures of the Study Director, Sponsor, Safety, and the Quality Systems Office.

IV.1.7: Exposure Concentration(s):

Solid test materials will be made into a paste with saline or distilled water. If saline or distilled water cannot be used, an appropriate non-toxic and non-irritating vehicle will be used. Acceptable alternatives include: gum Arabic, ethanol and water, carboxymethyl cellulose, polyethylene glycol, glycerol, vegetable oil, and mineral oil.

IV.1.8: Exposure Duration:

An exposure duration of four hours was selected with 4 ml samples taken at 1, 30, 60, 120 and 240 minutes. Samples will be processed by the Directorate of Laboratory Sciences and the rate of dermal absorption for each chemical will be determined.

IV.2. Data Analysis:

Concentrations of test article in receiver fluid will be compared between treatment and control groups using the t-test. Data will be used to determine the permeability coefficient (K_p) of the test substances through the stratum corneum.

V. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

Staff Member	Procedure	Training	Experience	Qualifications
McCain	Skin Preparation System Operation	DuPont (Dr. Bill Fasano) VMRCVM*^ Logan Inst. Co.	30+ yrs. Animal Research	Ph.D. Toxicology
Reddy	Advisor Sr. Scientist		40+ yrs. Research Including dermal penetration studies	PhD Toxicology DABT
Williams	Co-Investigator			
Hanna	Assist PI and Co-PI	USAPHC		
Way	Assist PI and Co-PI	USAPHC		BS Biology

*VA-MD Regional College of Veterinary Medicine

** Edgewood Chemical and Biological Center

***U.S. Army Center for Health Promotion and Preventive Medicine

^ Training was informal and conducted by qualified personnel

VI. BIOHAZARD/SAFETY:

Normal adherence to standard chemical and tissue handling procedures will be required during the performance of these studies. IMX-101, NTO, DNAN, NG and TNT will be considered a potentially hazardous materials and handled in accordance with SOP No. 83 "Health and Safety of Laboratory Personnel" (reference 11). Serological testing of tissue will be conducted by NDRI, this testing typically screens for the following: HIV I/II, HBV antigen and core antibody, HCV, syphilis, and may also include screens for CMV, HTLV I and II, and West Nile virus. NDRI does not knowingly ship tissues harboring infectious diseases (exception, NDRI's HIV research program).

VII. STUDY TIME FRAME:

VIII.1. Estimated Experimental Initiation Date:

October 2011

VIII.2. Estimated Experimental Completion Date:

December 2011

VIII. ASSURANCES: The law specifically requires several written assurances from the Study Director/Principal Investigator. Please read and sign the assurances as indicated.

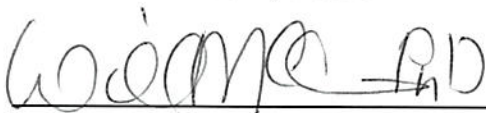
As the Study Director/Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

B. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis for scientific validity.

C. Training: I verify that the personnel performing the procedures / manipulations / observations described in this protocol are technically competent and have been properly trained.

Wilfred C. McCain, Ph.D.

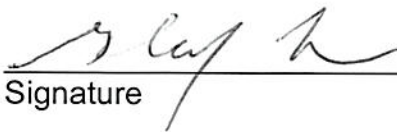


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Signature

Date

Glenn Leach, PhD, DABT
Manager, Toxicity Evaluation Program (85)



20130227

Signature

Date

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APPENDIX D

RAW DATA

SKIN IMPEDANCE VALUES FOR STUDY

INDIVIDUAL COMPOUNDS

NTO Impedance

	S-100		S-1000		P-100		P-1000	
Sample #	Start	24-Hours	Start	24-Hours	Start	24-Hours	Start	24-Hours
1	2.3	1.26	3	1.23	1.26	1.23	1.3	1.2
2	1.27	1.21	1.3	1.19	1.2	1.19	1.38	1.4
3	1.45	1.26	1.6	1.24	1.26	1.24	1.5	2.2
4	1.5	1.25	1.7	1.22	1.25	1.22	1.35	1.3
5	1.9	1.66	2.6	1.61	1.65	1.61	1.9	1.9
AVG	1.68	1.33	2.04	1.30	1.32	1.30	1.49	1.60
STDEV	0.41	0.19	0.72	0.18	0.18	0.18	0.24	0.43
STDER	0.17	0.08	0.30	0.07	0.08	0.07	0.10	0.18

DNAN Impedance

	S-100		S-1000		P-100		P-1000	
Sample #	Start	8-Hours	Start	8-Hours	Start	8-Hours	Start	8-Hours
1	2.02	2.04	1.91	1.87	2.06	2	1.95	1.91
2	1.63	1.62	1.55	1.54	1.63	1.62	1.55	1.54
3	1.47	1.46	1.4	1.38	1.48	1.47	1.4	1.38
4	1.66	1.63	1.58	1.55	1.66	1.63	1.59	1.55
5	1.56	1.7	1.49	1.61	1.56	1.7	1.49	1.62
6	1.62	1.63	1.56	1.55	1.63	1.63	1.56	1.55
AVG	1.66	1.68	1.58	1.58	1.67	1.68	1.59	1.59
STDEV	0.19	0.19	0.17	0.16	0.20	0.18	0.19	0.17
STDER	0.08	0.08	0.07	0.07	0.08	0.07	0.08	0.07

NQ Impedance

	S-100		S-1000		P-100		P-1000	
Sample #	Start	8-Hours	Start	8-Hours	Start	8-Hours	Start	8-Hours
1	2.47	1.74	1.85	1.66	2.33	1.74	1.9	1.66
2	1.72	1.49	1.51	1.4	1.79	1.49	1.54	1.4
3	1.68	1.39	1.46	1.31	1.66	1.39	1.47	1.31
4	1.53	1.35	1.41	1.28	1.57	1.36	1.42	1.28
5	1.62	1.32	1.4	1.23	1.61	1.33	1.42	1.24
6	1.48	1.47	1.67	1.32	1.69	1.4	1.49	1.31

AVG	1.75	1.46	1.55	1.37	1.78	1.45	1.54	1.37
STDEV	0.36	0.15	0.18	0.15	0.28	0.15	0.18	0.15
STDER	0.15	0.06	0.07	0.06	0.12	0.06	0.07	0.06

COMPOUNDS IN IMX-101

	S-100		S-1000		P-100		P-1000	
Sample		8-		8-		8-		8-
#	Start	Hours	Start	Hours	Start	Hours	Start	Hours
1	1.74	1.3	1.38	1.24	1.9	1.31	1.43	1.24
2	1.86	1.56	1.62	1.49	1.92	1.5	1.63	1.49
3	1.53	1.35	1.35	1.29	1.59	1.35	1.35	1.29
4	1.32	1.19	1.16	1.13	1.37	1.2	1.17	1.13
5	1.39	1.38	1.24	1.31	1.42	1.35	1.24	1.3
6	1.4	1.44	1.25	1.37	1.42	1.44	1.25	1.37
AVG	1.54	1.37	1.33	1.31	1.60	1.36	1.35	1.30
STDEV	0.22	0.13	0.16	0.12	0.25	0.10	0.17	0.12
STDER	0.09	0.05	0.07	0.05	0.10	0.04	0.07	0.05

COMPOUNDS IN IMX-101 UNDER PPE

	S-100		S-1000		P-100		P-1000	
Sample		8-Hours		8-		8-		8-
#	Start		Start	Hours	Start	Hours	Start	Hours
1	1.55	1.52	1.52	1.5	1.55	1.53	1.52	1.48
2	1.56	1.54	1.53	1.5	1.57	1.6	1.53	1.43
3	2.02	1.86	1.98	1.77	2.02	1.78	1.95	1.7
4	1.48	1.44	1.45	1.5	1.48	1.32	1.45	1.33
5	1.76	1.56	1.73	1.66	1.76	1.55	1.72	1.56
6	1.8	1.56	1.77	1.56	1.8	1.61	1.77	1.66
AVG	1.70	1.66	1.70	1.58	1.70	1.57	1.66	1.53
STDEV	0.20	0.20	0.20	0.11	0.20	0.15	0.19	0.14
STDER	0.08	0.08	0.08	0.05	0.08	0.06	0.08	0.06

INDIVIDUAL ABSORPTION VALUES FOR IMX-101 COMPONENTS

Table 1. Individual absorption values for NTO (100 mg) exposed to human epidermal tissue for 8 hours

NTO

Concentration	1-hr	2-hrs	4-hrs	6-hrs	8-hrs
NTO 1A	320	550	1600	1100	2800
NTO 2A	180	370	820	1300	3300
NTO 3A	99	230	600	890	2800
NTO 4A	100	240	470	940	2500
NTO 5A	140	360	880	1200	2200
*AVG	167.8	350	874	1086	2720
SDEV	91.40	129.42	438.27	172.28	408.66
STDER	75.25	156.95	391.93	487.00	1219.73
STDER ug/cm2	117.57	245.24	612.39	760.93	1905.83
+AVG ug/cm2	262.19	546.88	1365.63	1696.88	4250.00
AVG ug/cm2/hr	262.19	273.44	341.41	282.81	531.25

*Average values of penetration of skin area of 0.64 cm

+ Average values calculated per cm²

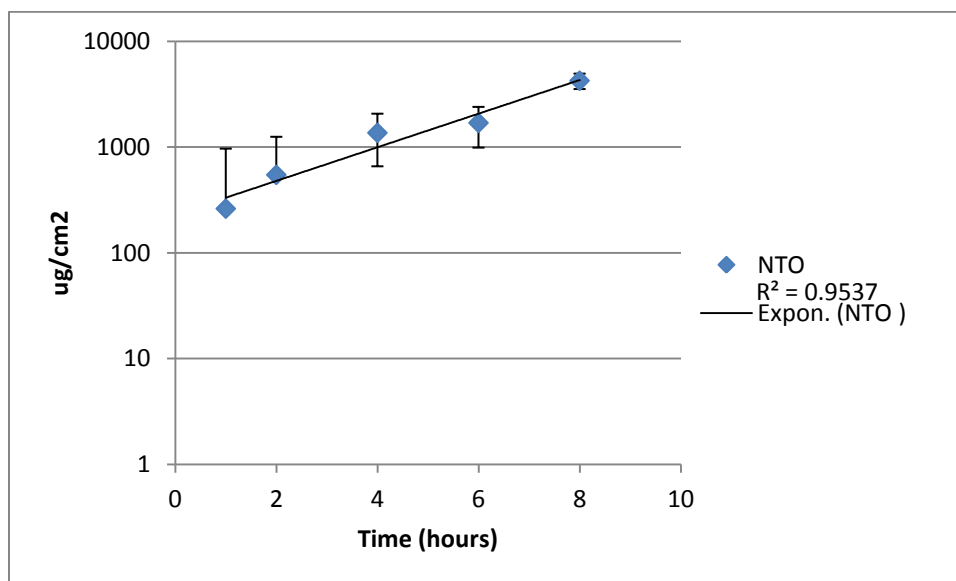


Figure 1. Cumulative absorption of NTO (100 mg) through human epidermal tissue during an 8-hour exposure

Table 2. Individual absorption values for DNAN (100 mg) exposed to human epidermal tissue for 8 hours

DNAN

Concentration	1-hr	2-hrs	4-hrs	6-hrs	8-hrs
DNAN 1A	1.7	1.9	0.67	11	15
DNAN 2A	1.7	0.2	0.39	0.2	7.1
DNAN 3A	0.2	1.2	0.2	8.8	1.8
DNAN 4A	0.2	0.92	0.28	7.1	3.5
DNAN 5A	0.2	0.76	0.37	6.4	9.5
DNAN 6A	0.2	0.91	0.38	7.8	11
*AVG	0.70	0.98	0.38	6.88	7.98
SDEV	0.77	0.56	0.16	3.64	4.89
STDER	0.29	0.40	0.16	2.81	3.26
STDER ug/cm2	0.45	0.63	0.24	4.39	5.09
+AVG ug/cm2	1.09	1.53	0.60	10.76	12.47
AVG ug/cm2/hr	1.09	0.77	0.15	1.79	1.56

*Average values of penetration of skin area of 0.64 cm

+ Average values calculated per cm²

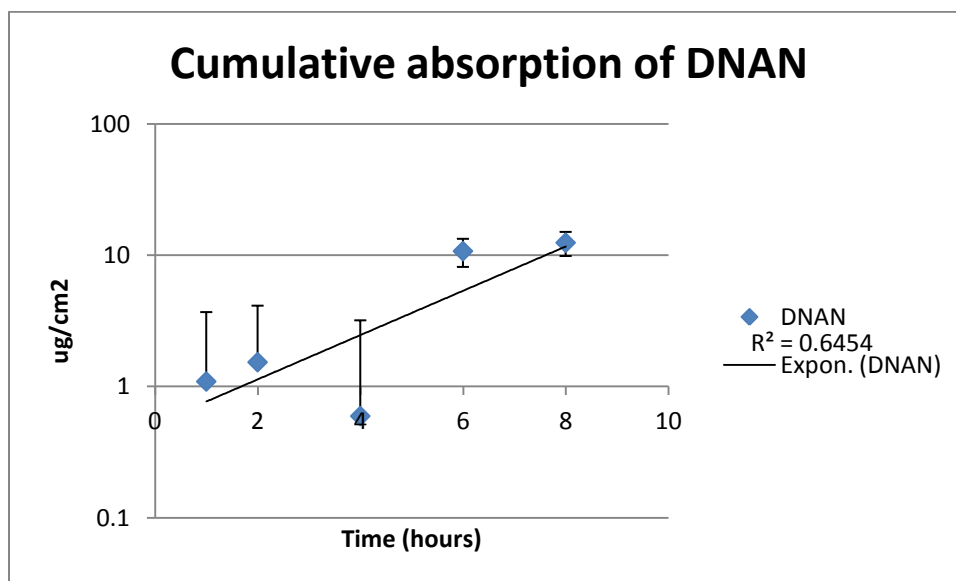


Figure 2. Cumulative absorption of DNAN (100 mg) through human epidermal tissue during an 8-hour exposure

Table 3. Individual absorption values for NQ (100 mg) exposed to human epidermal tissue for 8 hours

NG Concentration	1-hr	2-hrs	4-hrs	6-hrs	8-hrs
NQ 1A		19	75	130	230
NQ 2A		18	69	110	OR1
NQ 3A		21	74	150	210
NQ 4A		21	82	160	230
NQ 5A		12	66	130	OR ¹
NQ 6A		21	88	170	230
*AVG		18.67	75.67	141.67	225.00
SDEV		3.50	8.16	22.29	10.00
STDER		7.62	30.88	57.82	91.84
STDER ug/cm2		11.90	48.26	90.35	143.49
+AVG ug/cm2		29.17	118.23	221.35	351.56
AVG ug/cm2/hr		14.58	29.56	36.89	43.95

*Average values of penetration of skin area of 0.64 cm

+ Average values calculated per cm²

¹ out of range

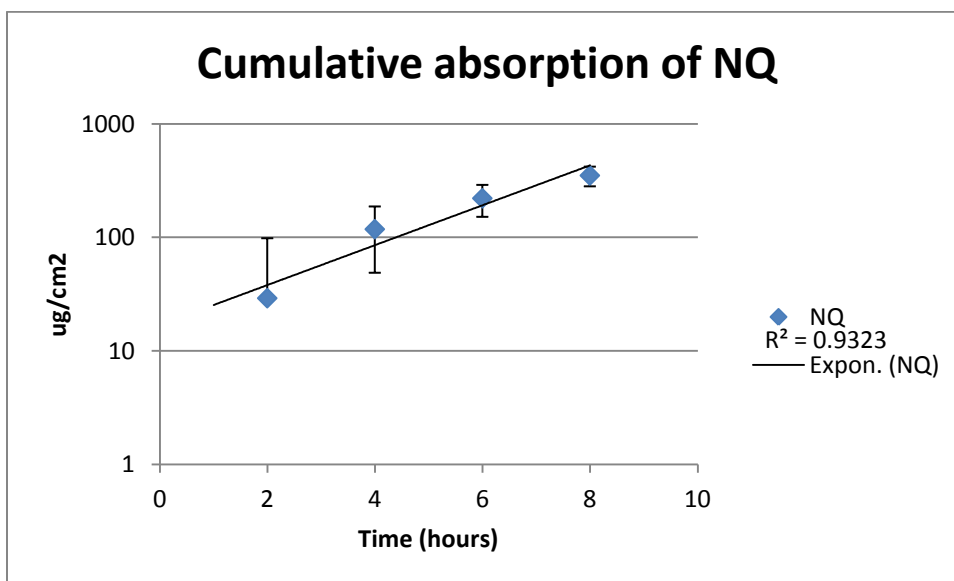


Figure 3. Cumulative absorption of NQ (100 mg) through human epidermal tissue during an 8-hour exposure

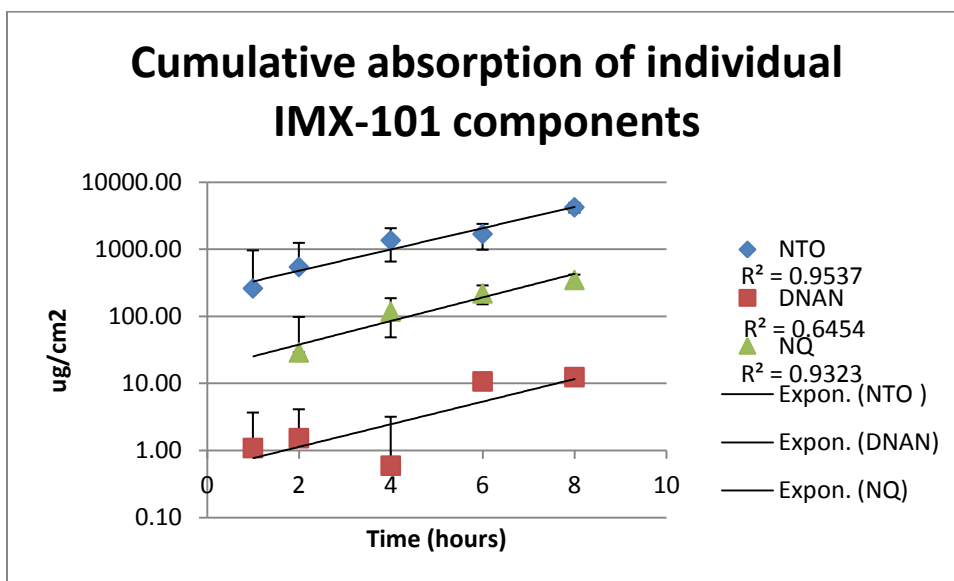


Figure 4. Cumulative absorption of individual IMX-101 components (100 mg) through human epidermal tissue during an 8-hour exposure

Table 4. Absorption of NTO as a component of IMX-101(100 mg) through human epidermal tissue during an 8-hour exposure

NTO					
Concentration	1 hr	2 hrs	4 hrs	6 hrs	8 hrs
NTO 1B	16	62	110	180	260
NTO 2B	100	150	350	340	400
NTO 3B	58	120	250	310	330
NTO 4B	120	240	490	660	810
NTO 5B	100	200	600	720	1000
NTO 6B	110	270	640	790	1000
*AVG	84.00	173.67	406.67	500.00	633.33
SDEV	39.46	77.82	207.14	253.85	342.33
STDER	34.29	77.88	182.36	224.22	284.01
STDER ug/cm2	53.57	121.68	284.94	350.34	443.76
+AVG ug/cm2	131.25	271.35	635.42	781.25	989.58
AVG ug/cm2/hr	131.25	135.68	158.85	130.21	123.70

*Average values of penetration of skin area of 0.64 cm,

+ Average values calculated per cm².

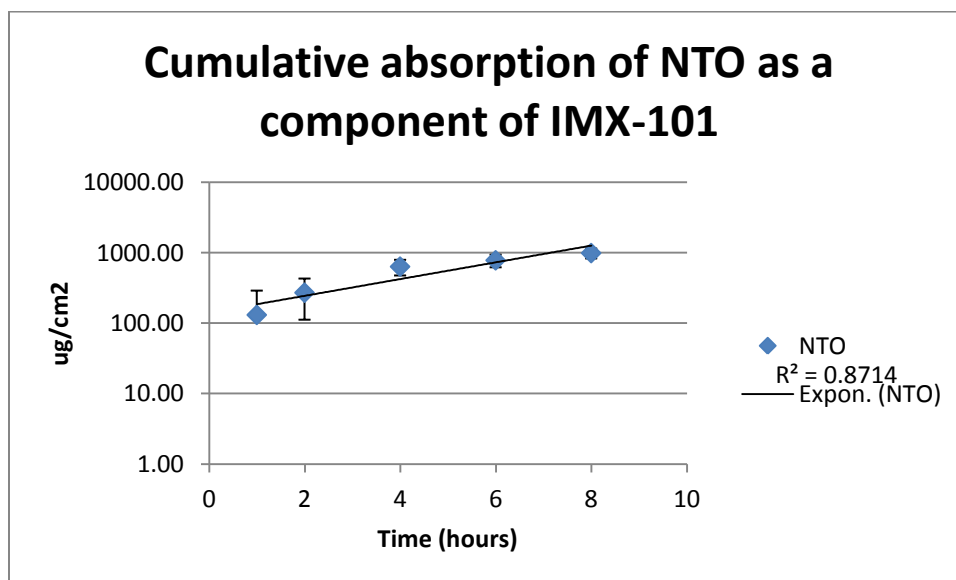


Figure 5. Cumulative absorption of NTO as a component of IMX-101 (100 mg) through human epidermal tissue during an 8-hour exposure

Table 5. Absorption of DNAN as a component of IMX-101 (100 mg) through human epidermal tissue during an 8-hour exposure

DNAN

Concentration	1 hr	2 hrs	4 hrs	6 hrs	8 hrs
DNAN 1B	0.00	0.00	0.61	1.60	4.50
DNAN 2B	0.51	2.20	4.50	7.00	11.00
DNAN 3B	0.00	7.10	4.50	5.30	8.20
DNAN 4B	0.61	2.40	5.80	9.90	13.00
DNAN 5B	0.52	1.70	5.70	10.00	14.00
DNAN 6B	0.58	3.40	7.50	12.00	16.00
*AVG	0.37	2.8	4.77	7.63	11.12
SDEV	0.29	2.38	2.32	3.80	4.20
STDER	0.15	1.26	2.14	3.42	4.99
STDER ug/cm2	0.24	1.96	3.34	5.35	7.79
+AVG ug/cm2	0.58	4.38	7.45	11.93	17.37
AVG ug/cm2/hr	0.58	2.19	1.86	1.99	2.17

*Average values of penetration of skin area of 0.64 cm

+ Average values calculated per cm²

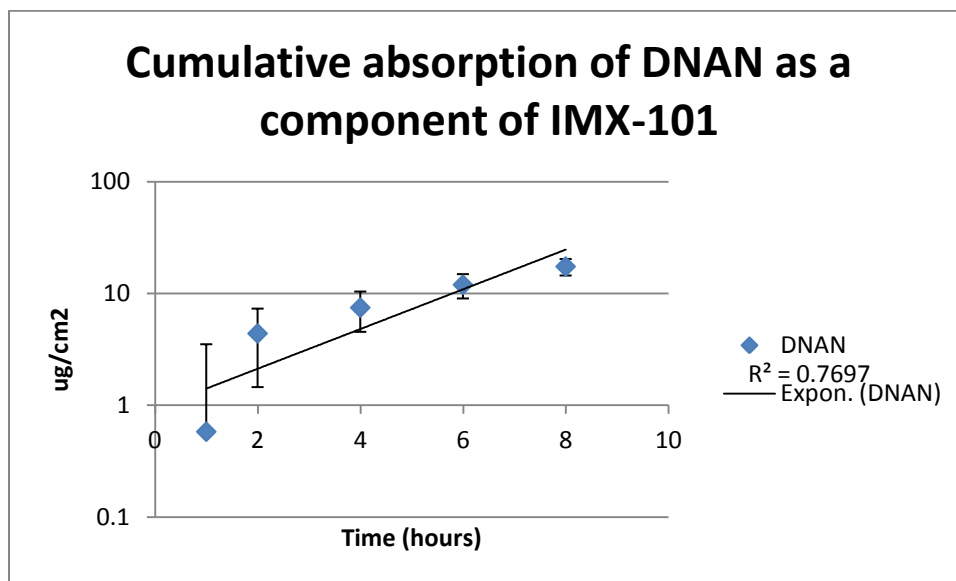


Figure 6. Cumulative absorption of DNAN as a component of IMX-101(100 mg) through human epidermal tissue during an 8-hour exposure

Table 6. Absorption of NQ as a component of IMX-101(100mg) through human epidermal tissue during an 8-hour exposure

NQ					
Concentration	1 hr	2 hr	4 hr	6 hr	8 hr
NQ 1B	720	1200	800	810	780
NQ 2B	440	280	480	490	560
NQ 3B	210	210	280	330	330
NQ 4B	170	190	320	370	510
NQ 5B	230	200	310	370	540
NQ 6B	140	190	300	400	540
*AVG	318.3333	378.3333	415.00	461.67	543.33
SDEV	223.38	403.95	201.97	178.93	143.48
STDER	129.93	169.66	186.10	207.03	243.65
STDER ug/cm2	203.02	265.09	290.78	323.48	380.70
+AVG ug/cm2	497.40	591.15	648.44	721.35	848.96
AVG ug/cm2/hr	497.40	295.57	162.11	120.23	106.12

*Average values of penetration of skin area of 0.64 cm,
+ Average values calculated per cm².

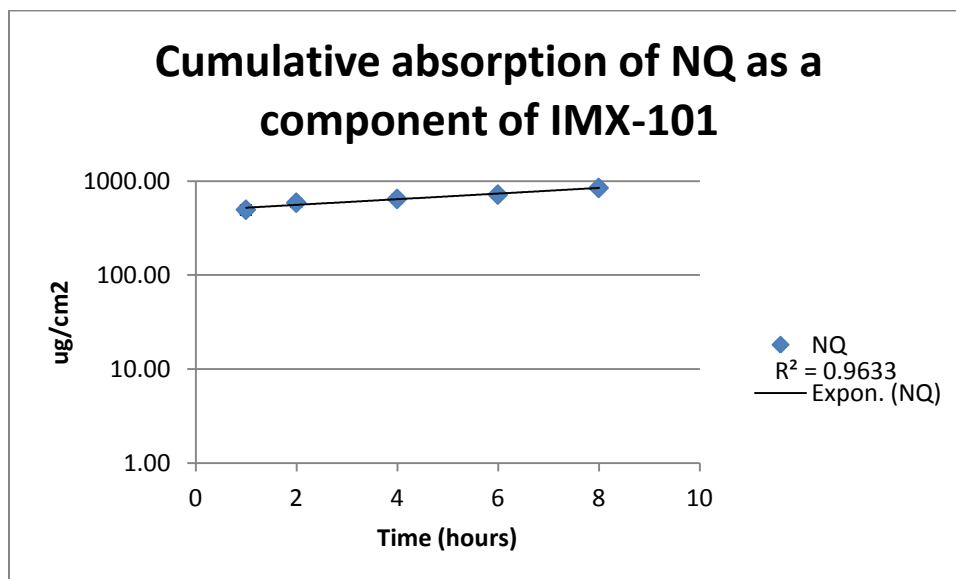


Figure 7. Cumulative absorption of DNAN as a component of IMX-101(100 mg) through human epidermal tissue during an 8-hour exposure

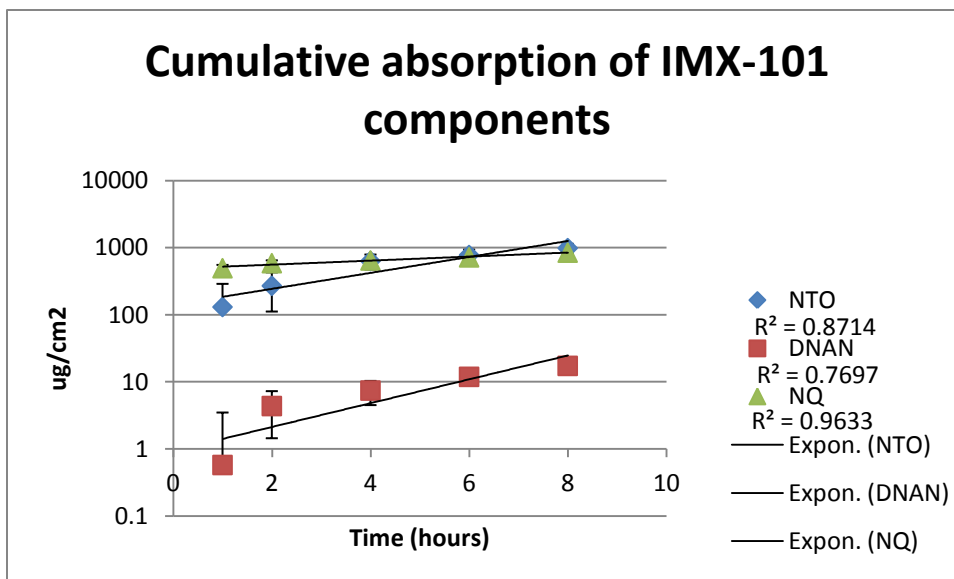


Figure 8. Cumulative absorption of combined IMX-101 components through human epidermal tissue during an 8-hour exposure

ABSORPTION OF IMX-101 COMPONENTS APPLIED UNDER PPE (TYVEK)

Table 7. Absorption of NQ as a component of IMX-101 (10 mg) through human epidermal tissue when applied under Tyvek during an 8-hour exposure

NTO

Concentration	1 hr	2 hrs	4 hrs	6 hrs	8 hrs
NTO 1C	59	150	150	200	260
NTO 2C	110	180	240	220	240
NTO 3C	340	230	310	360	400
NTO 4C	110	150	230	240	290
NTO 5C	60	120	220	290	260
NTO 6C	34	110	220	150	150
*AVG	118.83	156.67	228.33	243.33	266.67
SDEV	112.52	43.67	51.15	73.39	80.91
STDER	48.50	63.95	93.20	99.32	108.84
STDER ug/cm2	75.79	99.91	145.62	155.19	170.07
+AVG ug/cm2	185.68	244.79	356.77	380.21	416.67
AVG ug/cm2/hr	185.68	122.40	89.19	63.37	52.08

*Average values of penetration of skin area of 0.64 cm

+ Average values calculated per cm²

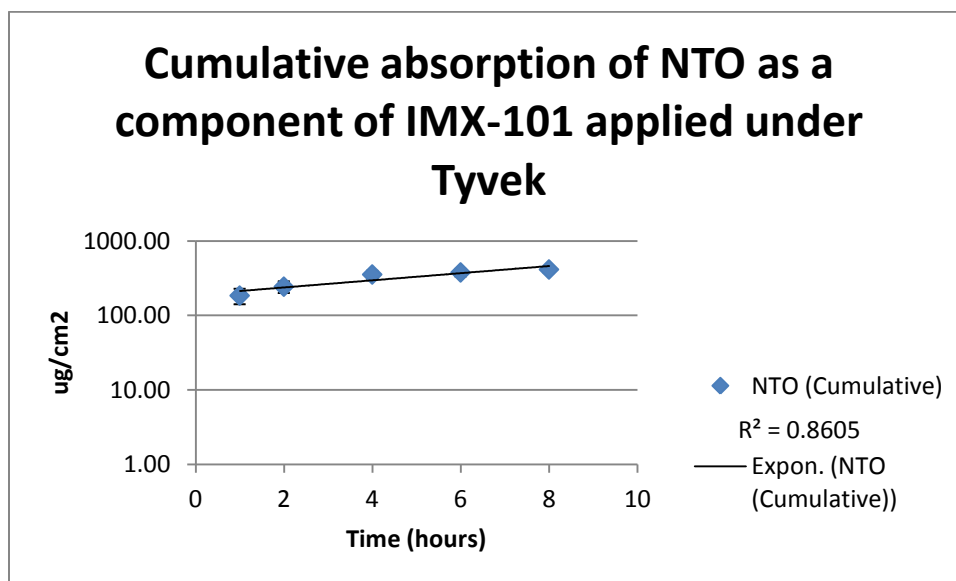


Figure 9. Cumulative absorption of NTO as a component of IMX-101 (10 mg) through human epidermal tissue when applied under Tyvek during an 8-hour exposure

Table 8. Absorption of DNAN as a component of IMX-101 (10 mg) through human epidermal tissue when applied under Tyvek during an 8-hour exposure

DNAN

Concentration	1 hr	2 hrs	4 hrs	6 hrs	8 hrs
DNAN 1C	0.87	2.2	4.5	6.6	12
DNAN 2C	1.8	3.6	6.5	9.5	14
DNAN 3C	1.6	2.3	4.3	6.7	11
DNAN 4C	1.3	2.3	4.9	7.1	11
DNAN 5C	0.82	1.9	4.8	6.9	10
DNAN 6C	0.83	2.2	5.3	5.2	6.6
*AVG	1.20	2.42	5.05	7.00	10.77
SDEV	0.43	0.60	0.79	1.40	2.45
STDER	0.49	0.99	2.06	2.86	4.39
STDER ug/cm2	0.77	1.54	3.22	4.46	6.87
+AVG ug/cm2	1.88	3.78	7.89	10.94	16.82
AVG ug/cm2/hr	1.88	1.89	1.97	1.82	2.10

*Average values of penetration of skin area of 0.64 cm

+ Average values calculated per cm²

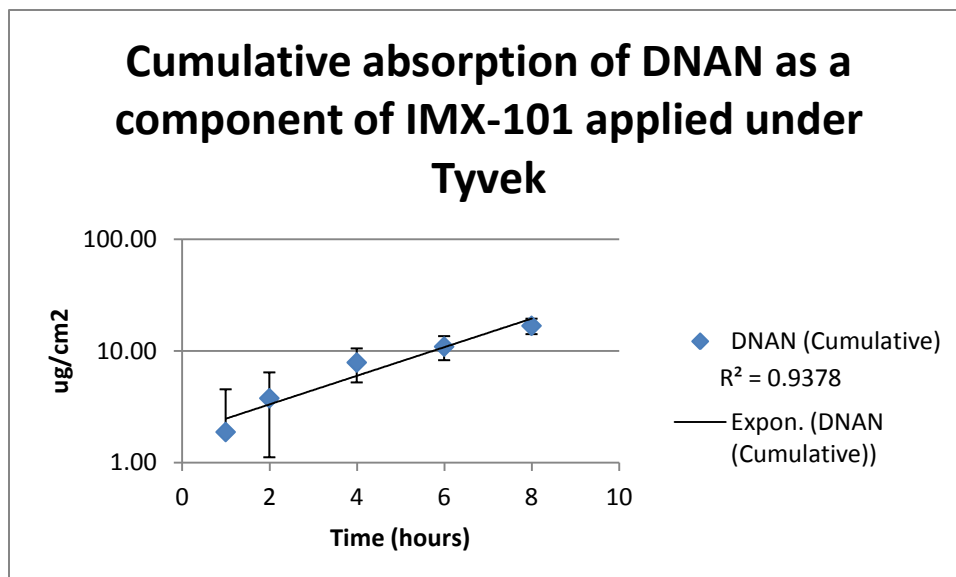


Figure 10. Cumulative absorption of DNAN as a component of IMX-101 (10 mg) through human epidermal tissue when applied under Tyvek during an 8-hour exposure

Table 9. Absorption of NQ as a component of IMX-101(10mg) through human epidermal tissue when applied under Tyvek during an 8-hour exposure

NQ					
Concentration	1 hr	2 hrs	4 hrs	6 hrs	8 hrs
NQ 1B	23	56	69	140	200
NQ 2B	46	73	160	190	240
NQ 3B	9	130	140	200	240
NQ 4B	95	66	110	140	210
NQ 5B	21	60	110	160	170
NQ 6B	14	46	100	66	110
*AVG	34.67	71.83	114.83	149.33	195.00
SDEV	32.18	29.92	31.75	47.86	49.30
STDER	14.15	29.32	46.87	60.95	79.59
STDER ug/cm2	22.11	45.81	73.24	95.24	124.36
+AVG ug/cm2	54.17	112.24	179.43	233.33	304.69
AVG ug/cm2/hr	54.17	56.12	44.86	38.89	38.09

*Average values of penetration of skin area of 0.64 cm

+ Average values calculated per cm²

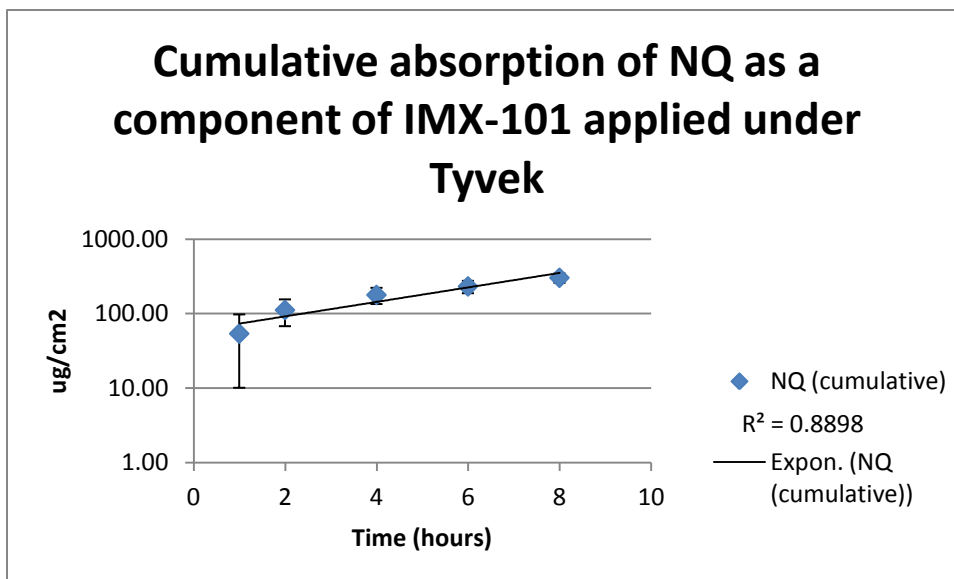


Figure 11. Cumulative absorption of NQ as a component of IMX-101(10 mg) through human epidermal tissue when applied under Tyvek during an 8-hour exposure

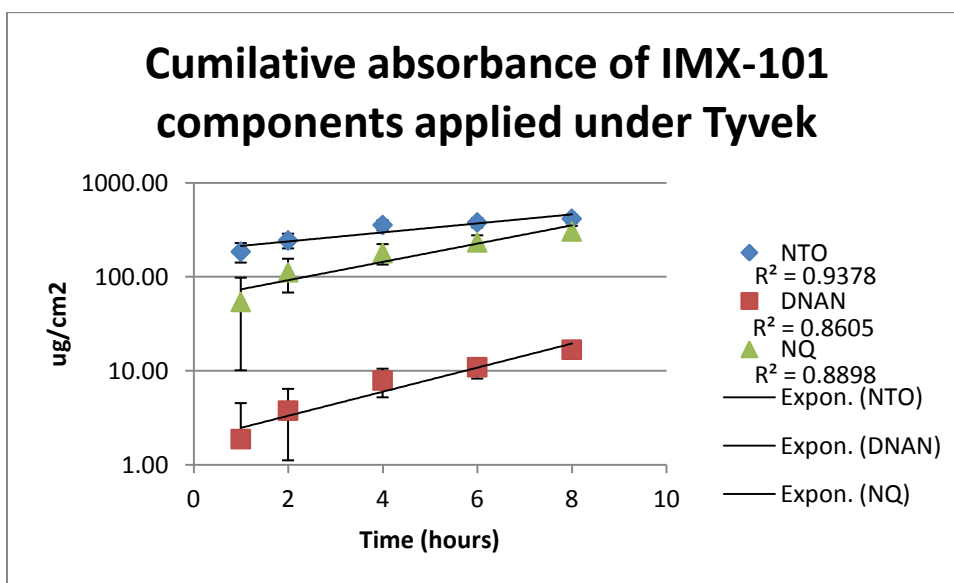


Figure 12. Cumulative absorption of IMX-101 components through human epidermal tissue when applied under Tyvek during an 8-hour exposure

Table 10. Comparative hourly absorption rate ($\mu\text{g}/\text{cm}^2$) of IMX-101 components under varying conditions when exposed to human epidermal tissue for 8 hours

	INDIVIDUAL	COMBINED	UNDER PPE
NTO	338.22	135.94	102.50
DNAN	1.07	1.768	1.93
NQ	31.24	236.28	46.42

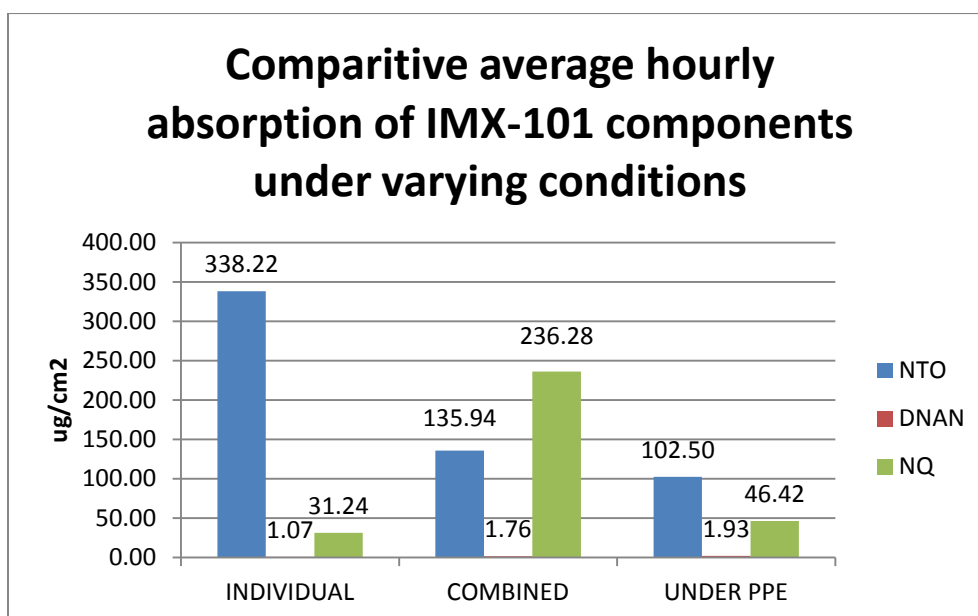


Figure 13. Comparative hourly absorption rate ($\mu\text{g}/\text{cm}^2$) of IMX-101 components under varying conditions when exposed to human epidermal tissue for 8 hours

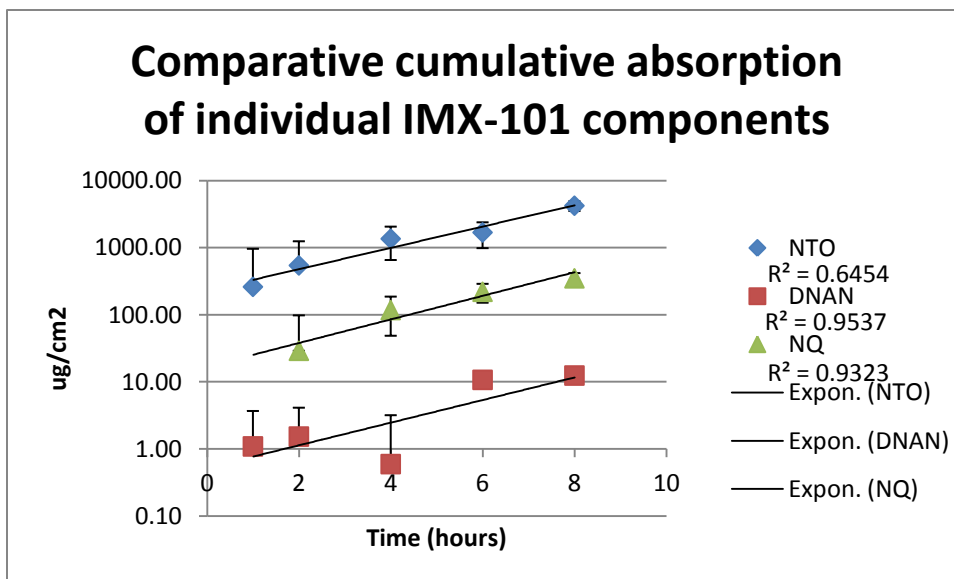


Figure 14. Comparative cumulative absorption of individual IMX-101 components through human epidermal tissue during an 8-hour exposure

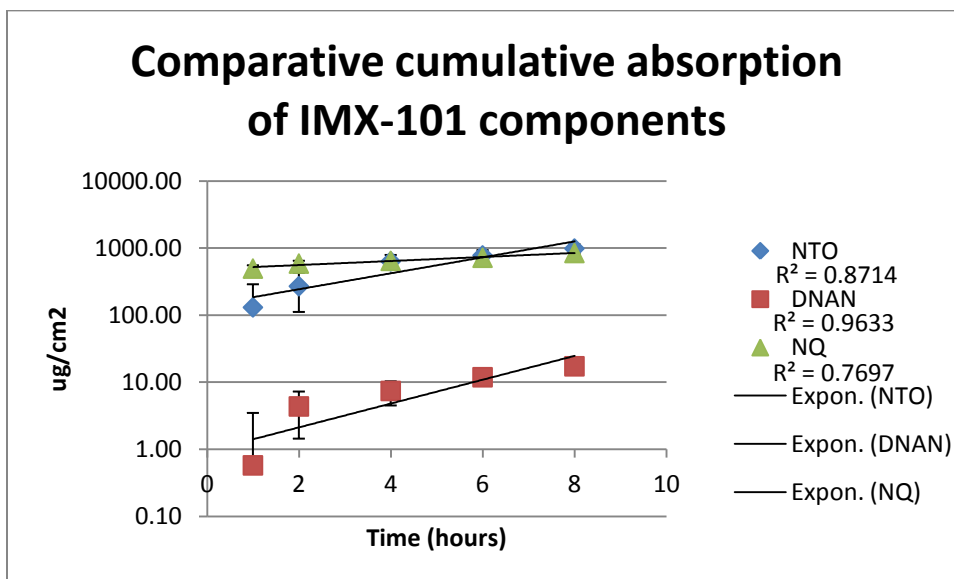


Figure 15. Comparative cumulative absorption of combined IMX-101 components through human epidermal tissue during an 8-hour exposure

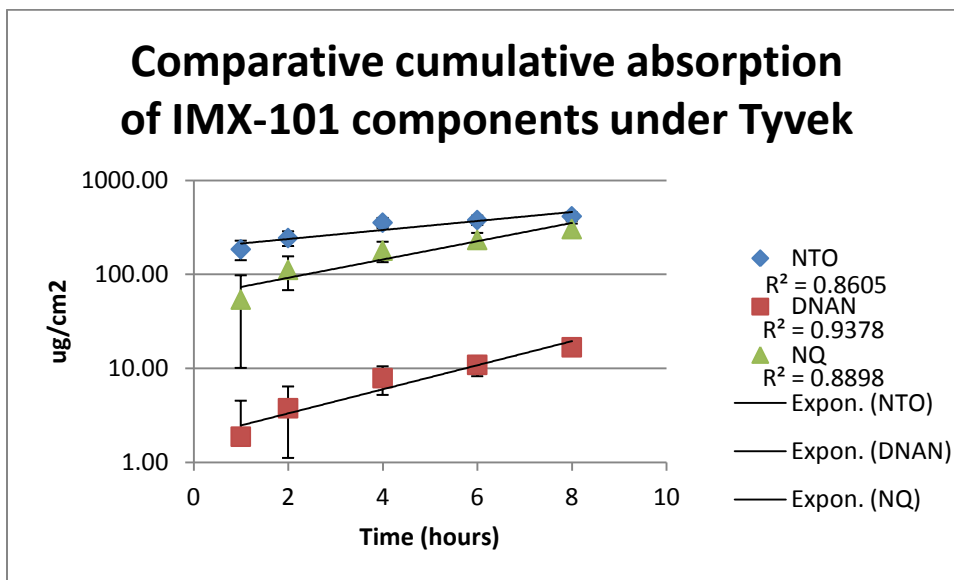


Figure 16. Comparative cumulative absorption of combined IMX-101 components through human epidermal tissue during an 8-hour exposure

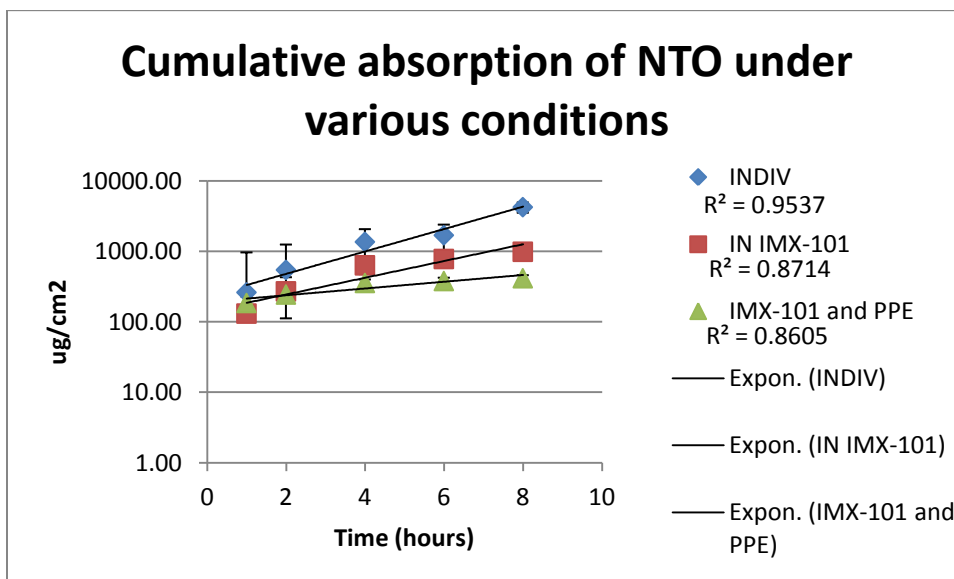


Figure 17. Comparative cumulative absorption of NTO through human epidermal tissue during an 8-hour exposure under varying conditions

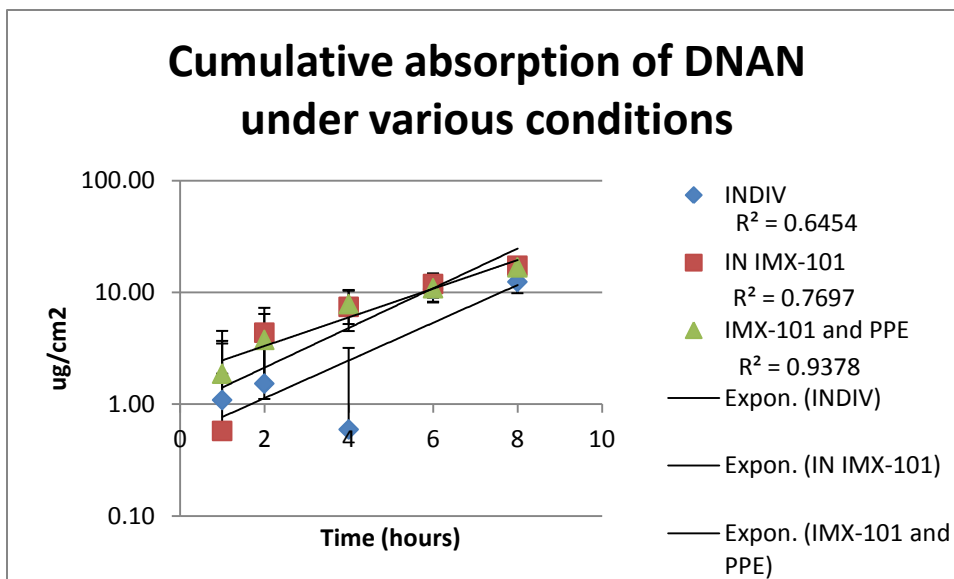


Figure 18. Comparative cumulative absorption of DNAN through human epidermal tissue during an 8-hour exposure under varying conditions

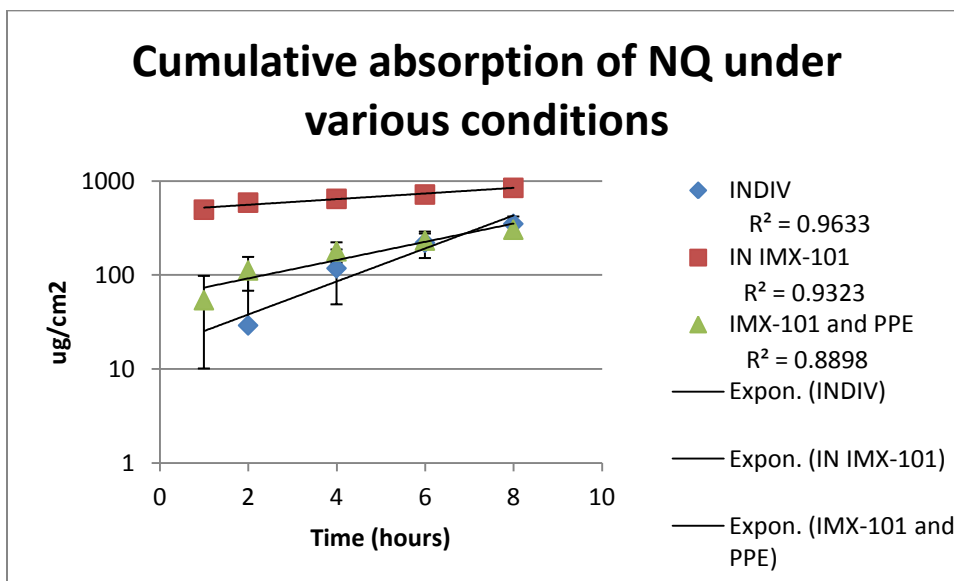


Figure 19. Comparative cumulative absorption of NQ through human epidermal tissue during an 8-hour exposure under varying conditions

Appendix E

Archives and Study Personnel

E-1. Archives

All raw data, documentation, records, protocols, and a copy of the final report generated as a result of this study will be archived in the storage facilities of the Toxicology Portfolio, AIPH, for a minimum of five (5) years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

Records on the test system will be archived by the Toxicology Portfolio for a minimum of five (5) years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

The present study used the laboratory protocol number 08UG-70-iv09-06-01o for all filings.

The protocol, raw data, summary data, and the final report pertaining to this study will be physically maintained within Building E-2100, AIPH. These data may be scanned to a computer disk. Scanned study files will be stored electronically in Room 3010, Building E-2100, USAIPH, Aberdeen Proving Ground (APG), MD, 21010.

Archived SOPs and maintenance and calibration logbooks may be found in Room 1026, Building E-2100, AIPH, APG, MD, 21010.

Archivist: Martha Thompson

E-2. Personnel

Management: Mark Johnson, Ph.D., Toxicology Portfolio Director and Program Manager, Health Effects Research Program (HERP)

Study Director: Wilfred C. McCain, Ph.D., Senior Toxicologist, T.E.P.

Quality Assurance: Michael P. Kefauver, Chemist, Quality Systems Office.