REPORT DOCUMENTATION PAGE

AFRL-SR-AR-TR-05-

0424

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
02/08/2005	Final Performance Report	06/01/2002-05/30/2005

4. TITLE AND SUBTITLE
Potentiation of Noise-Induced Hearing Loss by JP-8 Jet Fuel

5a. CONTRACT NUMBER
02-NL-023

5b. GRANT NUMBER
F49620-02-1-0274

5c. PROGRAM ELEMENT NUMBER
N/A

6. AUTHOR(S)
Randle M. Gallucci, Ph.D.
Laurence D. Fechter, Ph.D.
Jay Hanas, Ph.D.

5d. PROJECT NUMBER
N/A

5e. TASK NUMBER
N/A

5f. WORK UNIT NUMBER
N/A

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Oklahoma Health Sciences Center
Pharmaceutical Sciences/Pharmacy
P.O. Box 26901
OKC, OK 73190

8. PERFORMING ORGANIZATION REPORT
C1011101

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFOSR/NL
801 N. Randolph St., Ste. 732
Arlington, VA 22203-1977

10. SPONSOR/MONITOR'S ACRONYM(S)
DEPSCOR/AFOSR/NL

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
02-NL-023

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approve for Public Release: Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Military personnel are exposed to JP-8 through the skin, and the DoD has identified that one of the main complaints of personnel exposed to jet fuel is "skin problems" (www.JP-8.org). The purposes of this project were to assess potential hearing loss associated with JP-8 jet fuel exposures and loud noise, and also to describe the pathology of jet fuel dermatitis. A rat model of dermal exposure was chosen but found to be unsuitable to assess hearing loss, yet well suited to study skin inflammation. A small amount of JP-8 jet fuel or acetone control was applied to the skin of rats once a day for up to seven days. Skin samples were collected at various times, and inflammation was readily apparent in skin from JP-8 treated rats. JP-8 induced a number of inflammatory substances known as cytokines. Expression patterns of these cyokines were analyzed and showed that interleukin 1 and other cytokines associated with short-lived dermatitis dominated those associated with allergic dermatitis. Additionally, one cytokine closely associated with skin healing, interleukin 6 (IL-6) appeared to be depleted in the skin of JP-8 treated rats. It is possible that the reduction of IL-6 may inhibit healing, and partially explain the severity of the JP-8 dermatitis.

15. SUBJECT TERMS
JP-8, Jet fuel, dermatitis, inflammation, cytokines, hearing

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE		9	Randle M. Gallucci, Ph.D.
					19b. TELEPHONE NUMBER (include area code) 405-271-6593

Introduction:

JP-8 is a relatively new jet fuel that the Air force adopted for use in 1996. The Department of Defense (www.JP-8.org) has recognized JP-8 as the "single largest chemical exposure for its personnel". As documented on this DoD website, roughly 60 billion gallons of JP-8 are produced annually for all uses including jets, heaters and stoves, tanks etc.

Hearing loss constitutes one of the principal service-related injuries found among Veterans. Consequently, the VA spends over $440 million dollars per year in direct compensation for hearing loss (<http://chppm-ww.apgea.army.mil/hcp/comp_reports.aspx>).

The DOD has identified that one of the main complaints of personnel exposed to the JP-8 fuel is "skin problems" (www.JP-8.org). However, very little is known about this affliction. In addition to hearing loss, the secondary focus of this project was to determine the mechanisms of JP-8 induced dermatitis. We have found that numerous factors are produced in rat skin following exposure to JP-8 that could be the root source of dermatitis associated with jet fuel.

Accomplishments/New Findings:

The initial goals for this proposal were to examine the effects of jet fuel and noise on hearing loss in rats, and to detail the inflammatory response in JP-8 exposed rat skin. To assess hearing loss, rats were exposed to JP-8 alone by dermal exposure, an octave band of noise designed to produce a very limited degree of permanent hearing loss in a specific frequency region of the rats' audiogram, both agents in combination, and no experimental treatment. Pure-tone auditory thresholds were assessed 4 weeks later in order to measure permanent impairments in hearing. Unfortunately, while initial results seemed encouraging, skin exposure to JP-8 did not appear to be a suitable model for assessing the effects of jet fuel on hearing loss. Further experiments were initiated to examine hearing loss in an inhalation exposure model combined with noise, These were thought to provide more robust and consistent data. However, the apparatus available were neither accurate nor precise enough to determine the effects of JP-8 and noise on hearing loss. Thus, this specific aim could not be persued further as suitable apparatus could not be acquired within the timeframe of the contract.

Unlike the previous specific aim, the determination of the inflammatory processes in JP8 dermatitis proved much more fruitful. Additionally, further studies were performed that assessed the expression of stress proteins as markers of damage in various organs from rats exposed to JP8 through the skin. These investigations produced two peer reviewed publications and three abstracts presented at national meetings, and provide insight into the mechanisms that contribute to the acute yet severe nature of JP-8 contact dermatitis. Results are summarized below.

Model: Dorsal neck skin of rats was clipped, and 300 ul of JP-8 jet fuel was applied to denuded skin once a day for up to seven days. Twenty-four hours after either 1, 3, 5, or 7 days of exposure, skin samples from the exposed area were acquired by punch biopsy, then flash frozen in liquid nitrogen for determination of gene expression *via* either RT-PCR, real-time quantitative PCR, or ELISA, or preserved in 10% buffered formalin for histology.

Results: *Seven day repeated exposures:* Results for seven days repeated exposure were presented in an article published in the journal *International Immunopharmacology*, in September of 2004 (see below). Briefly, histology of skin biopsies showed mild to moderate inflammatory infiltrate, characterized primarily by neutrophils, eosinophils, and macrophages. The mRNA expression of a number of cytokines/chemokines were significantly induced in skin by JP-8 exposure including: IL-6, Eotaxin, Mip 1α, Mip-2, MCP-1, RANTES, and IP-10. Examination of cytokine expression patterns seemed to indicate that neutrophil chemotractant cytokines predominated those that would attract T Cells. Specifically, CXC/ELR+ chemokines such as GROα and β are associated with acute inflammation and PMN chemotaxis, whereas the ELR- type is associated with T cell accumulation and chronic inflammation. Following seven-day JP8 exposure there is a significant induction of the ELR+ CXC chemokines GROα (2 and 24 hours) and GROβ (24 hours), but not the ELR- chemokine IP10 (see fig 3, Gallucci, 2004). Increased ELR+ chemokine expression, and the lack of increased expression of ELR- type chemokines, may partially explain the transient nature of JP-8 induced irritant dermatitis. Thus, it seems that PMN chemotactic chemokines such as GROα might have a more profound effect on the pathogenesis of JP-8 skin dermatoses than those that would promote T cell sensitization. It was additionally found that of the primary inflammatory mediators, only IL-1 seemed to be significantly upregulated by JP-8 exposure. Thus, it seemed that IL-1 is the principal cytokine that initiates the inflammatory response, wereas TNFα plays only a minor role in JP8 dermatitis.

One, three, five, and seven-day exposures: Results from these experiments are currently being assembled, and

will be submitted for publication in the fall of 2005. Similar to what was determined in the investigations described above, the mRNA's of various CXC and CC chemokines were upregulated as a result of JP-8 exposure including Mip-2α and β (appendix, figure 1) and Mip1α (appendix, figure 2). IL-1β, and IL-6 mRNA's were significantly induced from 1 to 7 days, while IL-10 was not significantly different from control (appendix, figure 3). However, skin IL-6 protein content, as determined by ELISA, was not increased at any time point, and in fact was significantly decreased as compared to control after five and seven days of exposure (appendix, figure 4). This possibly indicated a direct or indirect post transcriptional modulation of this cytokine by jet fuel. This last observation could be significant, as IL-6 has been shown to be quite closely associated with skin wound healing (see Gallucci, et al., FASEB J., 2001, and Lin, Z., et al., J Leukoc Biol. 2003). This suggests that the severity of JP-8 dermatitis could be partially a result of an inhibited healing process, and not simply the result of toxic damage.

Determination of stress proteins as a result of dermal JP-8 exposure: Results from these experiments have been submitted for publication (see below). Briefly, histology of various organs following dermal JP-8 exposure indicated that jet fuel induces morphological alterations suggesting significant stress in the heart lesser amounts in kidney and liver. Because heat shock proteins are induced by variety of toxic agents and are considered indicators of cellular stress, these proteins were hypothesized to provide biomarkers as well as mechanistic information about any systemic toxicity resulting from dermal exposures to JP-8 fuel. Immunoblot analysis of tissues revealed increased levels of the inducible Heat Shock Protein 70 (HSP70) in the heart, kidney, and liver after this dermal JP-8 exposure. This exposure also leads to increased levels of heme oxygenase-1 (HO-1/HSP32) in the liver. Additionally during this exposure, a negative regulator of inflammation, IκBα (inhibitor of NF-κB), was increased in the liver, slightly increased in the kidney, and not increased in the heart. Two regions of the rat brain were also examined and HSP70 and IκBα were increased in the cerebellum but not significantly increased in the cortex. This study indicates dermal JP-8 exposure causes systemic alterations that are associated with cytoprotective activities (e.g., in the liver) as well as potentially toxic mechanisms (heart and kidney).

Personnel Supported:

Name	position	dates	% effort
Laurence Fechter, Ph.D.	PI (02)	06/02-12/02	25%
Randle Gallucci, Ph.D.	Co-I (02), PI (03-04)	06/02-05/05	10%
Jason Larabee	Student	06/02-05/05	25%
Sijy O'Dell	Technician	09/02-04/04	50%
David Faulkner	Technician	01/04-06/04	100%
Eric Lee	Technician	09/04-05/05	50%
Bethany Mickle	Technician	11/04-05/05	100%

Publications:

Larabee, J.L., Cheung, J., Lerner, M.R., Lightfoot, S., Brackett, D.J., Gallucci, R.M., Hocker, J.R., and Hanas, J.S., Stress Induced in Heart and Other Tissues by Rat Dermal Exposure to JP-8 Fuel. *Cell Biology and Toxicology*, 2005 in press.

Gallucci, R.M., O'Dell, S.J., Rabe, D.M., Faulkner, D.B.,and Fechter, L.D., Cytokine expression during JP8 jet fuel induced irritant dermatitis in rats, *In. J. Immunopharm*, 2004 Sept;4(9):1159-1169.

Interactions:

Poster presentations:

Fechter, LD and Gallucci, R.M., Dermal exposure to JP8 jet fuel: Disruption of auditory function, and induction of dermatitis in rats. *Toxicologist*, Suppl., 2004.

Gallucci, R.M., O'Dell, S.K., Rabe, D., and Fechter, L.D., JP-8 jet fuel exposure induces inflammatory cytokines in rat skin, *FASEB J.* Suppl., abstract #4502, 2004.

Gallucci, R.M., O'Dell, S.K., Rabe, D., and Fechter, L.D., JP-8 jet fuel exposure induces inflammatory cytokines in rat skin, *Tox Sci, Suppl,* Abstract #476, 2005.

Meeting presentations:

Randle M. Gallucci, Sijy K. O'Dell, Daniel Rabe, and Laurence K. Fechter. JP-8 Jet Fuel Exposure Induces Inflammatory Cytokines in Rat Skin. *JP-8 Jet Fuel Exposure and Health Effects Meeting,* Tucson, AZ 2003.

Randle M. Gallucci, Sijy K. O'Dell, Daniel Rabe, and Laurence K. Fechter. JP-8 Jet Fuel Exposure Induces Inflammatory Cytokines in Rat Skin. *JP-8 Jet Fuel Exposure and Health Effects Meeting,* Tucson, AZ 2004.

New Discoveries/inventions/patents:

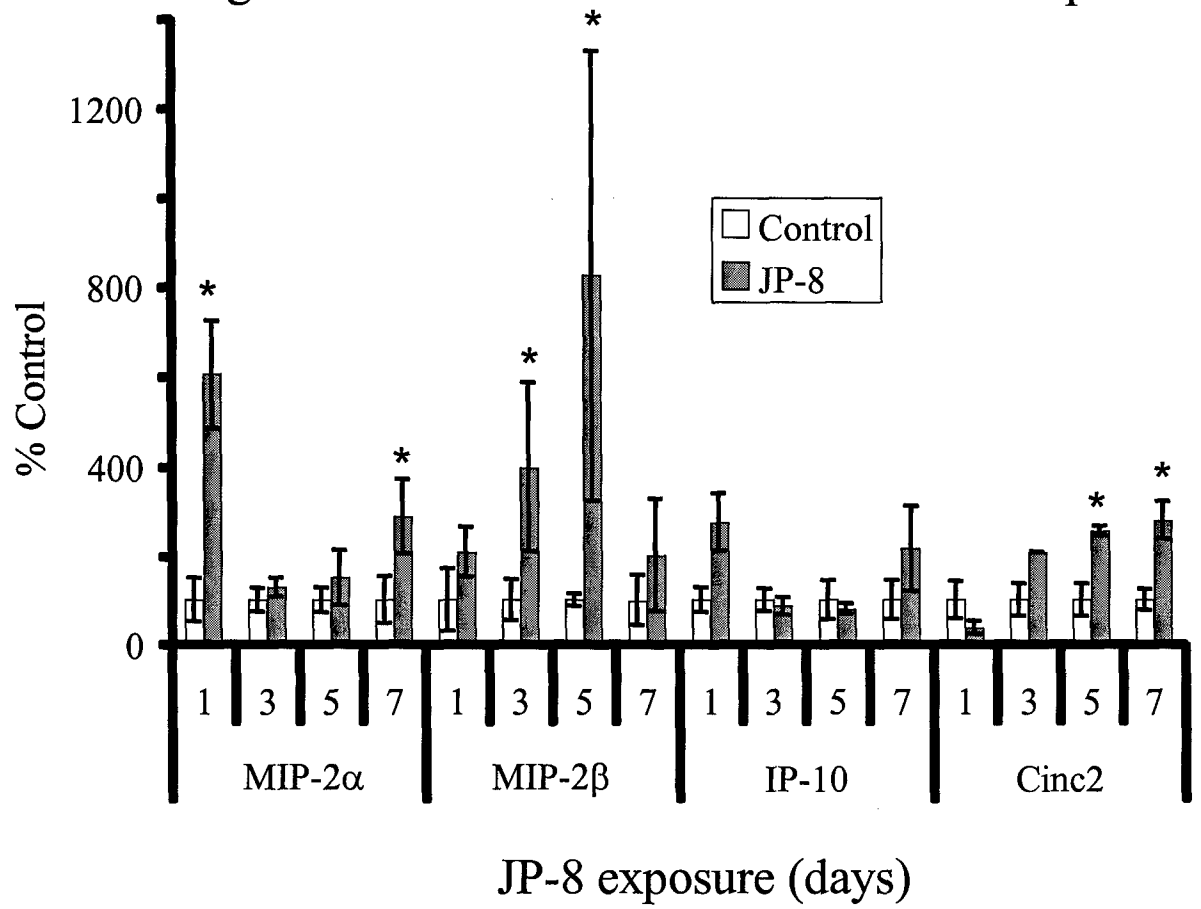
Scientific discoveries outlined above. No inventions or patents.

Honors Awards:

University of Oklahoma Health Sciences Center, Provost's Junior Faculty Research Award, Randle Gallucci, Ph.D. recipient, April, 2005.

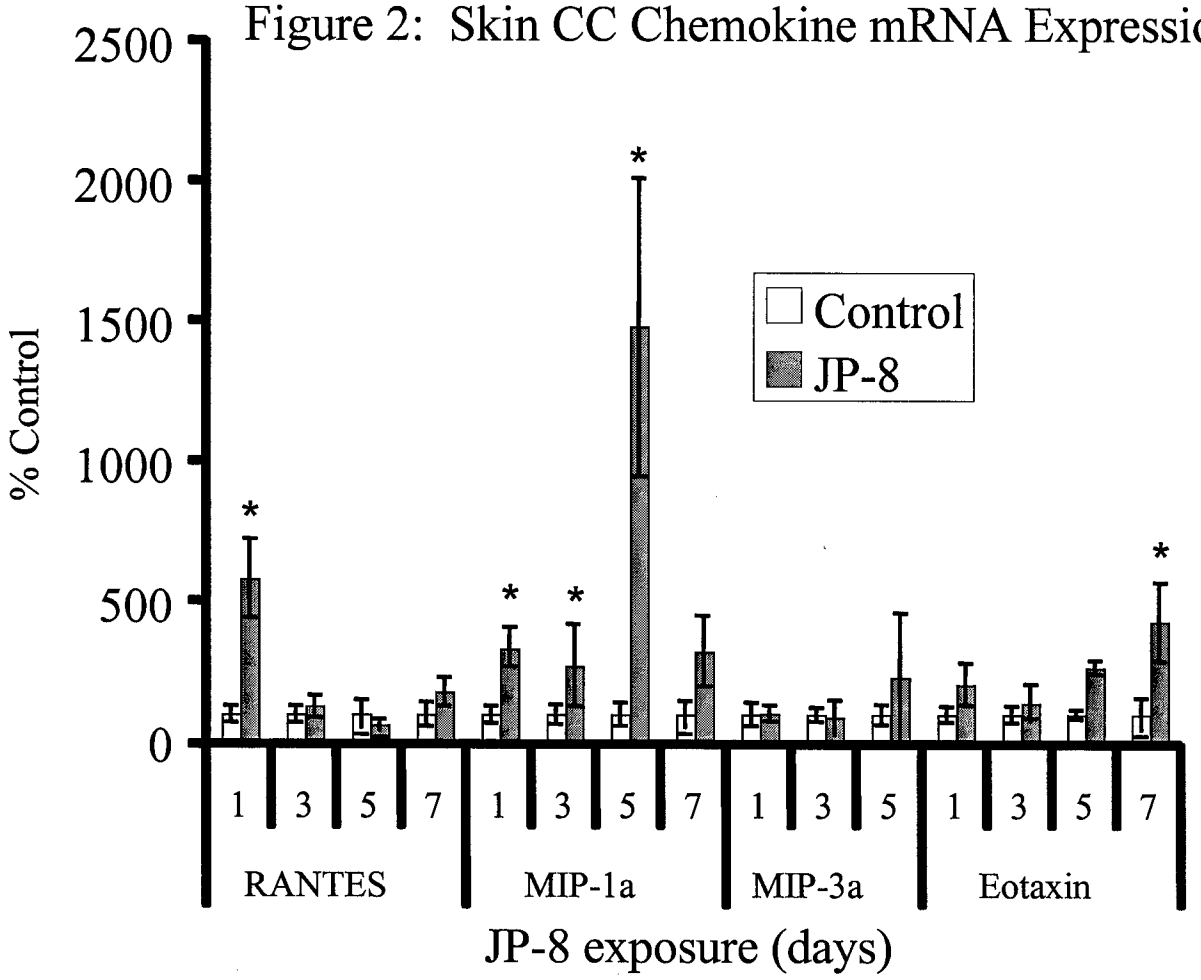
Appendix

Figure 1: Skin CXC Chemokine mRNA Expression



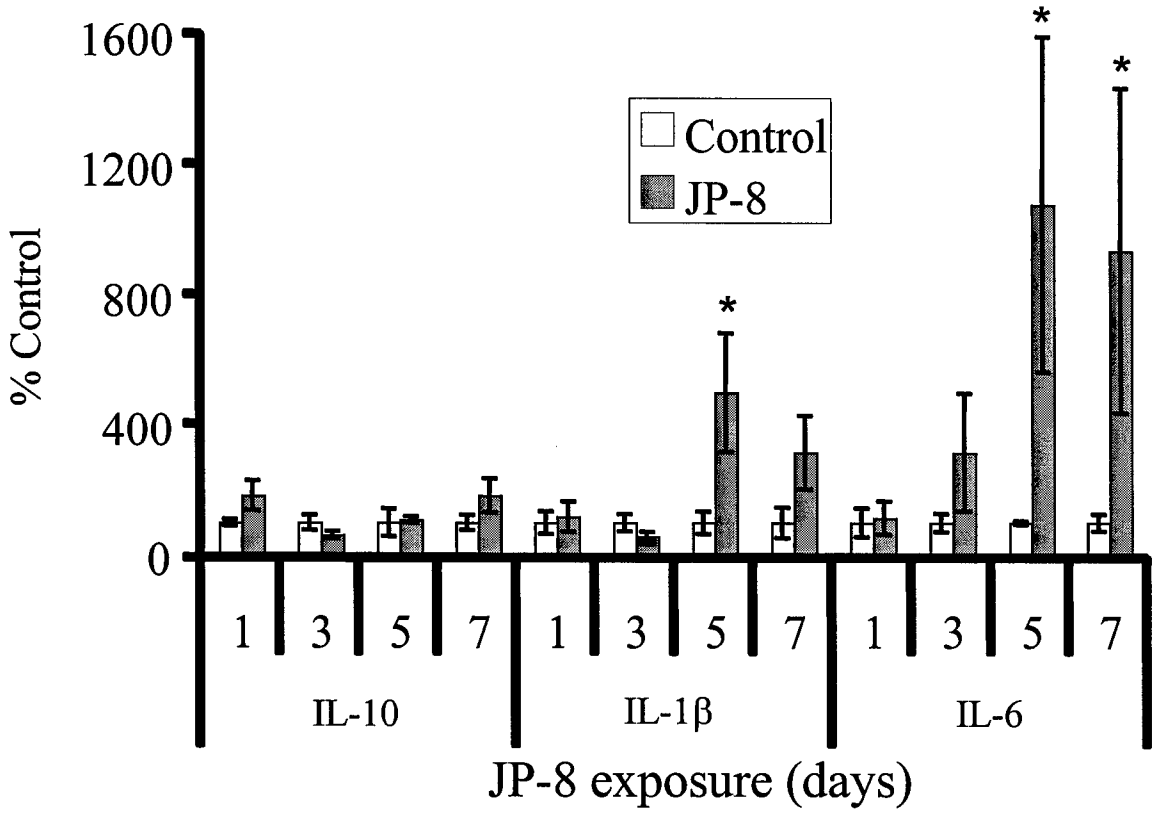
*Significantly different ($p \leq 0.05$) from respective acetone control

Figure 2: Skin CC Chemokine mRNA Expression



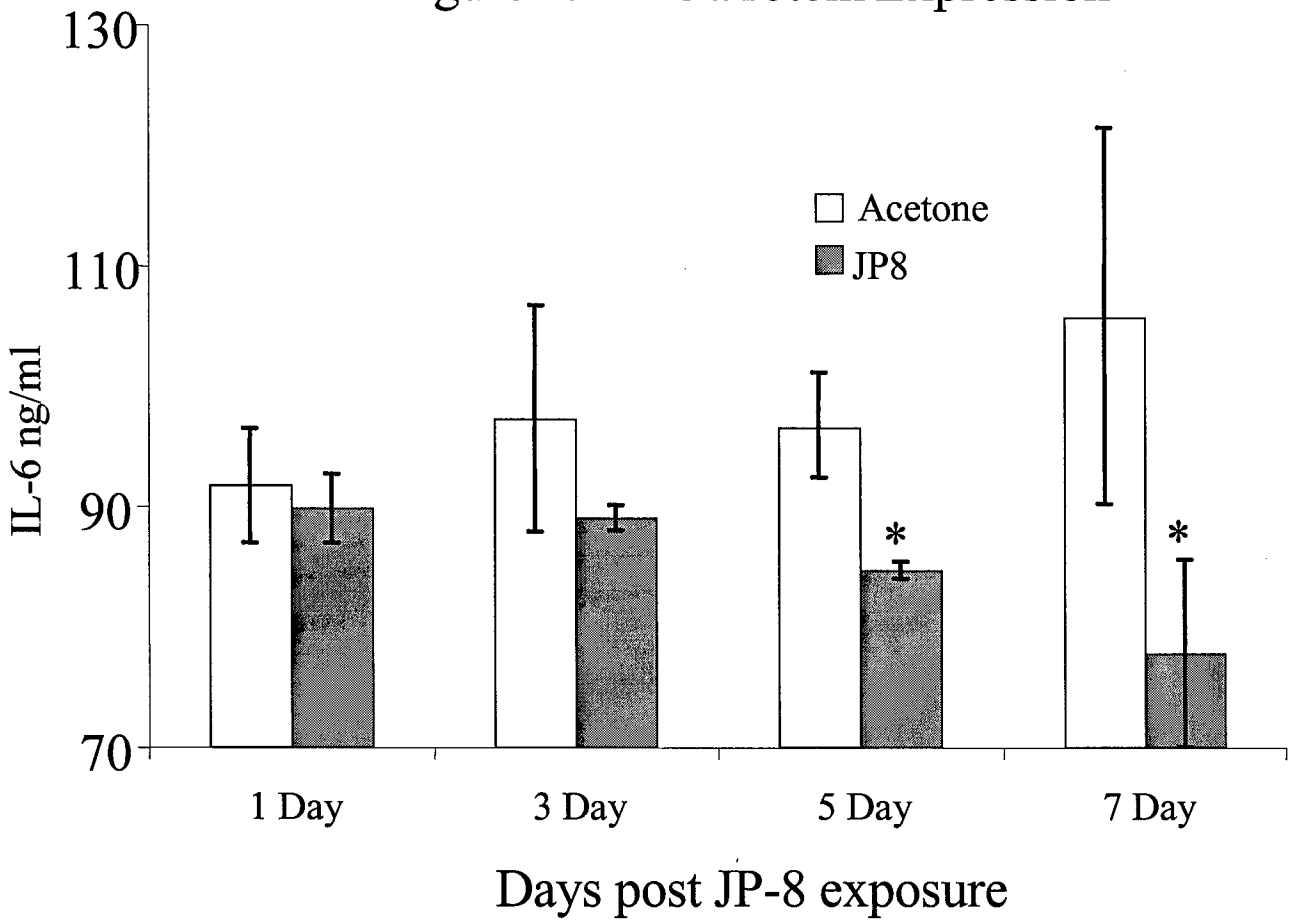
*Significantly different ($p \leq 0.05$) from respective acetone control

Figure 3: Skin Cytokine mRNA Expression



*Significantly different ($p \leq 0.05$) from respective acetone control

Figure 4: IL-6 Protein Expression



Significantly different ($p<0.05$) from: *day 1 JP8 skin, respective acetone control