



UV-B Exposure Impairs Resistance to Infection by *Trichinella spiralis*

Wim Goettsch,¹ Johan Garssen,¹ Anton Deijns,¹ Frank R. de Gruijl,² and Henk van Loveren¹

¹National Institute for Public Health and Environmental Protection, Bilthoven, the Netherlands; ²Department of Dermatology, Utrecht University, the Netherlands

To assess the possibility that increases in UV-B exposure on the earth's surface could lead to impaired resistance to several infectious diseases, we studied the effect of UV-B exposure on resistance against *Trichinella spiralis*. Wistar rats, orally infected with *T. spiralis* larvae, were exposed to suberythral doses of UV-B radiation daily for 5 days at different time periods before or after infection. A significant increase in the number of *Trichinella* larvae was found in the carcasses of rats irradiated with UV-B between 6 and 10 days after infection. These data indicate that exposure to UV-B radiation suppresses the resistance to a parasitic infection. We suggested that UV-B radiation especially suppresses cellular immune responses against these worms because specific IgM, IgG, and IgE titers were not significantly altered by UV-B exposure. These data indicate that UV-B irradiation plays a role in the course of infection with *T. spiralis*, which suggests that increases of UV-B exposure might also lead to problems with other infectious diseases and might affect vaccination because of the interaction of UV-B irradiation with memory T-cells. **Key words:** antibodies, immunosuppression, infectious diseases, ozone layer, *Trichinella spiralis*, ultraviolet-B radiation. *Environ Health Perspect* 102: 298–301(1994)

It is well known that a decrease in the thickness of the ozone layer may lead to higher exposure to ultraviolet radiation [especially ultraviolet-B (280–315 nm) radiation]. UV-B radiation induces the appearance of skin-associated diseases such as erythema (1), photoaging (2,3), tumors (4), and eye-associated diseases such as keratitis (5,6) and cataracts (7,8). In the case of nonmelanoma skin cancers (NSMC), it is calculated that (9–11) that a decrease of the ozone layer by 1% could lead to an increase of NSMC by 2.3%. It is also plausible that decreases of the ozone layer could lead to increases of cataracts of the eye (12,13).

In addition, the effects of UV-B exposure on the immune system have been studied for some time. In 1974, Kripke (14) stated that UV-B radiation could induce tolerance against antigenic UV-B-induced tumors of the skin. It was already

demonstrated that immunosuppression, such as in patients with organ transplants who had certain forms of immunosuppression therapy, could increase the incidence of NMSC. Later, experiments with mice showed that UV-B exposure impaired the skin immune response, not only locally but also systemically; suppression was found in places not exposed to UV-B (15–18). Immune responses in other parts of the body are also affected by UV-B exposure (19–21); however, these effects on non-skin-associated immune responses are very diverse and often contradictory.

UV-B can suppress the immunological resistance to skin infections such as *Leishmania* and *Candida albicans* (22,23) in mice. Moreover, it has been reported that UV-B exposure leads to the induction of systemic suppression of resistance against non-skin-associated infections in mice, such as *Mycobacterium bovis* (24). Infectious diseases are a much greater problem in tropical and subtropical areas than at higher latitudes. However, it is not justified to single out UV-B exposure from a list of possible causes (e.g., temperature, humidity, environmental conditions) when areas like Australia and the southern United States are considered, where infectious diseases are no great problem, in spite of comparatively high UV-B irradiance. Nevertheless, UV-B exposure can impair the activity of memory T-cells, which could lead to problems with the effectiveness of vaccinations, which, especially in poor countries, is the main way of controlling infectious diseases.

In immunotoxicology, host-resistance models in the rat are used to analyze the immunotoxic or -suppressive effects of

chemical compounds. One of these models is *T. spiralis*. *T. spiralis* is a parasite which may be present in raw meat. Not only in (sub)tropical areas, but also in Eastern Europe, this parasite is found in the human population and may be a hazard for the human health. We used *T. spiralis* to analyze the immunosuppressive effects of UV-B radiation. Our data indicate that UV-B radiation can suppress resistance to *T. spiralis*.

Male Wistar rats (SPF), 6–8 weeks old (RIVM, Bilthoven, the Netherlands), were housed in macrolon cages and were provided with commercial rat chow (Trouw, Nijkerk, the Netherlands) and tap water *ad libitum*. Each cage housed five animals. The rats were irradiated on four shaved spots (2.8 cm²) on the back with a Kromayer UV-lamp (Hanovia, Switzerland) for 16 sec every day, for five days (Table 1). The Kromayer lamp was chosen because the size and the location (e.g., the neck or the flank) of the skin exposed to UV-B is exactly known, which is important for the quantitative relation between UV-B exposure and the immunosuppressive effect. In addition, the spectrum of the Kromayer is more comparable to the spectrum of the sun compared to the spectrum of the FS40 lamp. In addition, this lamp is used in experiments with human volunteers, so data from rat and mice experiments may be compared to human experiments. Finally, it is easy to add several cut-off filters to the Kromayer lamp to obtain a wavelength action spectrum of the effect studied. The spectrum of the Kromayer lamp is shown in Figure 1. The spectrum, as determined with the Optronics OL-752-O-PMT, reveals that of this total UV irradiation, 3% is UV-C (250–280 nm), 45% is UV-B (280–315 nm), and 52% is UV-A (315–400 nm). The action spectrum for UV-induced suppression of contact hypersensitivity (25) indicates that UV-B and UV-C are potent immunosuppressive forms of radiation. The differences

Table 1. Exposure scheme

Group	Days of exposure ^a
1	Control
2	-4-0
3	1-5
4	6-10
5	11-15
6	16-20
7	21-25

^aDay 0 is day of infection.

Address correspondence to J. Garssen, National Institute for Public Health and Environmental Protection, PO Box 1, 3720 BA Bilthoven, the Netherlands.

This work was supported by the National Research Programme Global Change grant 850017 from the Ministry of Public Housing and Environmental Protection and Environmental Research Programme grant EV5V-CT910028 from the European Community. We thank H. de Rooij for his technical assistance and J.G. Vos for reviewing this manuscript.

Received 2 September 1993; accepted 28 December 1993.

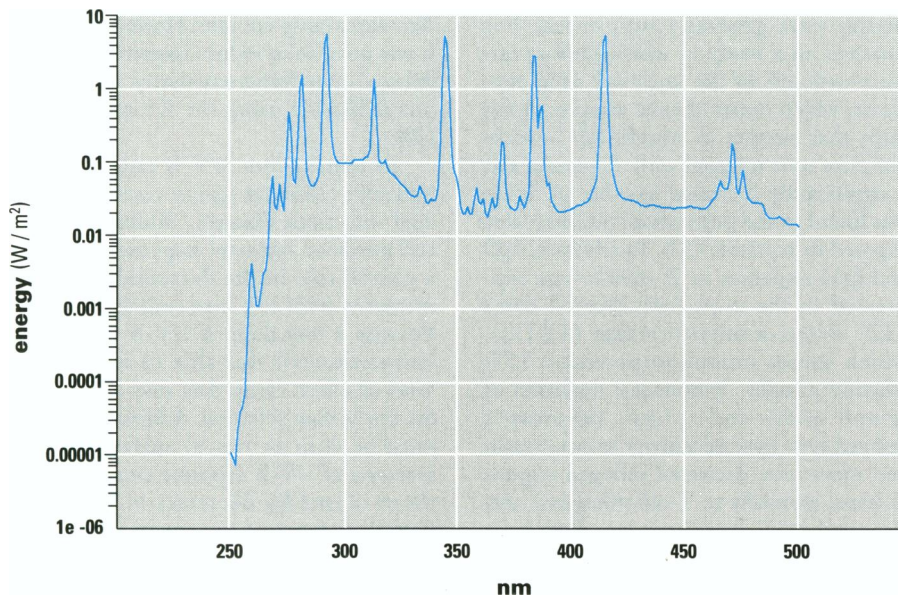


Figure 1. Wavelength spectrum (250–500 nm) of the Kromayer lamp. Spectrum determined with a Optronics OL-752-0-PMT spectrophotometer, using a monochromator (wavelength interval is 1 nm). Intensity is in watts/m².

between UV-B and UV-C are not large, and using the Kromayer lamp, UV-B will be mainly responsible for the immunosuppressive effect. The daily exposure of rats was 800 mJ total UV irradiation (4 spots \times 2.8 cm² \times 16 sec \times 4.48 mJ/cm²), which is equal to approximately 0.5 minimal erythema dose (MED). The controls were similarly shaved and handled as the UV-exposed rats (group 7), except for the actual irradiation (sham irradiation).

Rats were infected according a procedure described elsewhere (26). Briefly, rats were orally dosed with 1000 *T. spiralis* larvae in 0.5 ml phosphate-buffered saline (PBS). Rats were euthanized by CO₂ asphyxiation, skinned, and eviscerated. The carcasses were cut into 0.5-cm³ pieces. We mixed 100 g of this tissue in a bottle with 1 l of water. We then added 10 ml of HCl, 36% (Merck, Germany), and 7.5 g of pepsin (RIVM, Bilthoven, The Netherlands). This bottle was continuously stirred for 2 hr at 37°C. The suspension was divided into two portions and after 15 min the larvae were precipitated on the bottom. To remove bones and hair the larvae were filtered and washed in PBS.

We determined larvae according to procedures described elsewhere (26). Briefly, the larvae were stirred in a solution of 400 ml PBS for 5 min. Using a micropipet, we placed 10 0.05-ml drops on a petri dish. The total amount of larvae in these drops was counted with a microscope (40 \times). This procedure was performed in duplicate, and used to determine the number of larvae/milliliter. Finally, the total number of larvae in the carcass was determined using the following formula:

$$\text{larvae/carcass} = (\text{mean larvae in } 50 \mu\text{l} \times 8000)$$

At the day of the infection (day 0), and on days 14, 28, and 42, blood was collected from rats and analyzed for *Trichinella*-specific antibodies (IgG, IgM, and IgE). Cultures dishes (Greiner, Germany) were coated with *T. spiralis* antigen and the plates were incubated with the serum of the rats. After washing, we incubated the dishes with specific monoclonal mouse anti-rat IgM, IgG, and IgE (Sanbio BV Uden, the Netherlands), using peroxidase-conjugated affinity-purified rat anti-mouse IgG as a conjugate (Jackson Immuno-research Laboratories, West Grove, Pennsylvania). Substrate was added, and in combination with H₂SO₄ the extinction was determined at 450 nm.

We analyzed data for significant differences using one-way analysis of variance (ANOVA). All calculations were performed with the statistical software package Minitab (Minitab Inc., State College, Pennsylvania).

Figure 2 shows the larvae count in carcasses of infected rats after UV-B exposure. The average count was 1×10^5 larvae/carcass, which is in the normal range at 42 days after infection with 1000 *T. spiralis* larvae. It is clear that UV-B exposure at days 6–10 increases the amount of larvae in carcasses significantly ($p < 0.05$). The *Trichinella* antibody titers (especially IgE), determined 42 days after infection (Fig. 3), were elevated in groups 3, 4, and 5; however, these differences were not statistically significant. In contrast, a decrease in IgE titers was seen in groups 6 and 7, and the decrease in group 7 was statistically significant ($p < 0.05$).

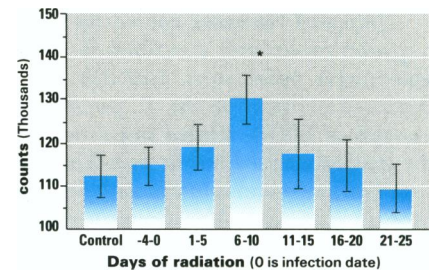


Figure 2. Number of *T. spiralis* larvae determined in the carcasses of rats after 42 days of infection. Groups are divided as described in text. The mean \pm SE of 10 animals per group is shown. *Significantly different from control, $p < 0.05$.

The results showed that a suberythral UV-B exposure given at days 6–10 after infection induced a higher number of *Trichinella spiralis* larvae in the carcasses of infected rats. At other time points UV-B failed to induce this significant increase in larvae. The suppression of resistance to *T. spiralis* caused by UV-B exposure was less severe compared to decreased resistance due to the congenital absence of a (cellular) immune response: In the carcasses of nude athymic rats (*nu/nu*), the yield of muscle larvae at day 49 after infection was 10 times higher than in normal (+/*nu*) rats (26).

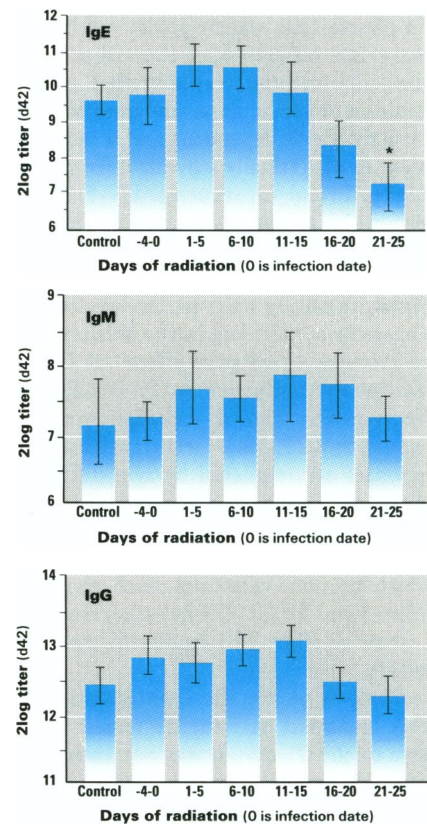


Figure 3. *T. spiralis*-specific antibody titers (IgE, IgM, and IgG) determined in the blood of rats after 42 days of infection. The antibody titer is presented as 2log titers. Groups are divided as described in text. The mean \pm SE of 10 animals per group is shown. *Significantly different from control, $p < 0.05$.

Decreased resistance caused by UV-B has been extensively described for skin-associated infections (22,23). The decreased resistance to the *T. spiralis* is an example of UV-B-induced impairment of the resistance to a systemic non-skin-associated infection and is in concordance with experiments with *Mycobacterium bovis* (24).

The timing of the UV-B exposure determines the severity of the inhibition of the resistance to this parasite. After ingestion of infected meat, larvae excyst in the acid pepsin environment of the stomach, enter the small intestine, and become sexually mature within 1–3 days. After copulation, the adult females penetrate the intestinal mucosa where they produce viviparous larvae. The newborn larvae migrate to host striated muscle via the lymphatics and blood vessels over the next 1–3 weeks and are encapsulated by host-derived fibrin. In rats, immunological specific inflammation of the bowel, which mediates expulsion of the worms from the gut, becomes evident at about 6 days after primary infection in rats. This response is characterized by the accumulation of intestinal mast cells, eosinophilic and polymorphonuclear granulocytes, lymphocytes, and plasma cells and by goblet cell hyperplasia and reduction of the villus:crypt ratio. The cellular response that causes expulsion of the adult worms from the gut is strictly T-cell dependent and is virtually absent in congenitally athymic mice or rats (26,27). In our experiments, UV-B radiation caused the most pronounced impairment of resistance when rats were UV-B irradiated at a stage of infection in which cellular immunity starts to develop. This is in agreement with data in the literature on the immunosuppressive effects of UV-B radiation that indicate that UV-B radiation affects predominantly cellular immune responses (28,29). Cellular immune responses are regulated by two different subsets of T-helper (Th) cells: Th1 cells (which produce cytokines, such as interferon- γ and interleukin-2) and Th2 cells (which produce cytokines, such as interleukin-4 and 5). UV-B irradiation especially seems to affect Th1-regulated responses (30,31).

Analysis of specific immunoglobulin titers indicated that the decreased resistance to the *T. spiralis* was not associated with decreased levels of specific immunoglobulins. In contrast, the amount of antibodies specific for *T. spiralis*, and especially IgE, seems to be increased after UV-B irradiation in animals showing reduced resistance to *T. spiralis* after UV-B exposure. In other studies, where decreased resistance to *T. spiralis* was encountered as result of immunosuppression, such anti-

bodies were generally suppressed. For instance, in a study in which mother rats were exposed to the antiviral drug acyclovir, which causes thymic atrophy in the pups that persists in adulthood, 6-week-old-pups were infected with *T. spiralis*, and a significantly increased number of larvae was found in the pups whose mothers were exposed to acyclovir (32). In addition, IgG and IgM responses to *T. spiralis* were suppressed in the pups from treated dams (32). Bis(tri-*n*-butyltin)oxide (TBTO), which causes immunosuppression (33) (thymus atrophy, lymphocyte depletion of lymph nodes and spleen, suppressed delayed-type hypersensitivity to ovalbumin and tuberculin, decreased mitogen responsiveness, especially to T-cell mitogens), was also used in *T. spiralis* studies. Resistance to *T. spiralis* was significantly suppressed as shown by retarded expulsion of adult worms from the small intestine, increased counts of muscle larvae (2.5 times higher than in controls) (33), and suppressed IgE titers (34). The use of these immunosuppressants causes an increased amount of muscle larvae in combination with suppressed IgE, in contrast with the UV-B experiments. Because IgE synthesis is T-cell dependent, and more precisely Th2 dependent, our data indicate that immunosuppression caused by UV-B exposure between days 6 and 10 after infection does not affect Th2-mediated responses that are required for IgE synthesis (35). Moreover, in experiments in which UV-B-treated mice were exposed to *Leishmania* (22), impaired delayed-type hypersensitivity to this parasite was observed. Delayed-type hypersensitivity responses are Th1-mediated (35). In addition, in the *Leishmania* model, UV-B failed to affect antibody production. More recent data suggest that the Th1 subset is important for resistance to parasites like *Leishmania* and *T. spiralis* (36). It has been described that UV-B radiation interacts with keratinocytes in the skin, resulting in release of several cytokines, such as interleukin-10 (37). Interleukin-10 impairs Th1-mediated immune responses but not Th2-mediated responses (35). Thus, we suggest that UV-B irradiation affects Th1-regulated immune responses, which results in a decreased resistance to *T. spiralis* worms, but does not affect the amount of *T. spiralis* specific antibodies, which is Th2-mediated. It is noteworthy that although exposure to UV-B between days 16 and 25 after infection does not influence larvae yields, IgE titers in these animals are decreased, which is significant in rats exposed to UV-B 21–25 days after infection.

Results on the induction of immunosuppression by UV-B exposure using the

Kromayer lamp are rare. However, in mice it was possible to induce impairment of the delayed-type hypersensitivity reaction to picrylchloride using the Kromayer lamp (38).

In conclusion, UV-B radiation can influence resistance against nonskin-associated infectious diseases. Inhibition of T-cell mediated immune responses may play a pivotal role in the decreased resistance measured with this host-resistance model. Because a low dose of UV-B irradiation (suberythral) was able to induce this kind of effect, these data may be relevant for the human situation. Additional studies must be done in rats to analyze whether increases of UV-B exposure comparable to those caused by decreases of the ozone layer have immunosuppressive activity and increase the severity of parasitic infection. In the future, these data, combined with data from noninvasive studies that determine the immunosuppressive activity of UV-B exposure in human population, could form a basis for risk estimation.

REFERENCES

1. Gange RW, Parrish JA. Acute effects of ultraviolet radiation upon the skin. In: Photoimmunology (Parrish JA, Kripke ML, Morison WL, eds). New York:Plenum Press, 1983;77–94.
2. Kligman AM. Perspectives and problems in cutaneous gerontology. *J Invest Dermatol* 73:39–46(1979).
3. Gilchrist BA, Szabo G, Flynn E, Goldwyn RM. Chronic and acutely induced aging in human facial skin. *J Invest Dermatol* 80S:81–85(1983).
4. Blum HF. Carcinogenesis by ultraviolet light. New Jersey:Princeton University Press, 1959.
5. Pitts DG, Kay KR. The photo-ophthalmic threshold for the rabbit. *Am J Optom* 46:561–572(1969).
6. Cogan DG, Kinsey VE. Action spectrum of keratitis produced by ultraviolet radiation. *Arch Ophthalmol* 35:660–677(1946).
7. Grover D, Zigman S. Coloration of human lenses by near-UV photooxidised tryptophan. *Exp Eye Res* 13:70–76(1972).
8. Lerman S. Radiant energy and the eye. New York:Macmillan, 1986.
9. Fears TR, Scotto J. Estimating increases in skin cancer morbidity due to increases in ultraviolet radiation exposure. *Cancer Invest* 1:119–126(1983).
10. Kellfens G, De Grijl FR, Van der Leun JC. Ozone depletion and increase in annual carcinogenic ultraviolet dose. *Photochem Photobiol* 52:819–823(1990).
11. McDonald JE. Relationship of skin cancer incidence to thickness of ozone layer. Statement submitted to hearings before the House Subcommittee on Transportation Appropriations, March 2, 1971. *Congressional Record* 117(39):3493(1971).
12. Madronich S, Bjorn LO, Ilyas M, Caldwell MM. Changes in biologically effective ultraviolet radiation reaching the earth's surface. In: Environmental effects of ozone depletion: 1991 update (van der Leun JC, Tevini M,

- eds). Nairobi:United Nations Environment Programme, 1991;1-13.
13. Maitchouk IF. Trachoma and cataract: two WHO targets. *Int Nurs Rev* 32:23-25(1985).
 14. Kripke ML. Antigenicity of murine skin tumors induced by ultraviolet light. *J Natl Cancer Inst* 59:1227-1230(1974).
 15. Kripke ML, Morison WL. Studies on the mechanism of systemic suppression of contact hypersensitivity by UVB radiation. II. Differences in the suppression of delayed and contact hypersensitivity in mice. *J Invest Dermatol* 86:543-549(1986).
 16. Noonan FP, Bucana C, Sauder DN, De Fabo HC. Mechanism of systemic immune suppression by UV irradiation. II. UV effects on number and morphology of epidermal langerhans cells and the UV-induced suppression of contact hypersensitivity have different wavelength dependencies. *J Immunol* 132:2408-2416(1984).
 17. Morison WL, Bucana C, Kripke ML. Systemic suppression of contact hypersensitivity by UVB radiation is unrelated to the UVB-induced alterations in the morphology and number of Langerhans cells. *Immunology* 142:299-306(1984).
 18. Hersey P, Haran G, Hasic E, Edwards A. Alteration of T cell subsets and induction of suppressor T cell activity in normal subjects after exposure to sunlight. *J Immunol* 31:171-174(1983).
 19. Gurish MF, Lynch DH, Daynes RA. Changes in antigen-presenting cell function in the spleen and lymph nodes of ultraviolet irradiated mice. *Transplantation* 33:280-284(1982).
 20. Letvin NL, Nepom JT, Greene MI, Benacerraf B, Germain RN. Loss of Ia-bearing splenic adherent cells after whole body ultraviolet radiation. *J Immunol* 125:2550-2554(1980).
 21. Tokura Y, Miyachi Y, Takigawa M, Yamada M. Ultraviolet-induced suppressor T cells and factor(s) in murine contact photosensitivity. I. Biological and immunochemical characterization of factor(s) extracted from suppressor T cells. *Cell Immunol* 110:305-320(1987).
 22. Giannini MSH. Suppression of pathogenesis in cutaneous leishmaniasis by UV-irradiation. *Infect Immunol* 51:838-846(1986).
 23. Denkins Y, Fidler IJ, Kripke ML. Exposure of mice to UV-B radiation suppresses delayed hypersensitivity to *Candida albicans*. *Photochem Photobiol* 49:615-619(1989).
 24. Jeevan A, Kripke ML. Alteration of the immune responses to mycobacterium bovis (BCG) in mice exposed chronically to low doses of UV radiation. *Cell Immunol* 145:2087-2091(1989).
 25. De Fabo EC, Noonan FP. Mechanism of immune suppression by ultraviolet irradiation in vivo. I. Evidence for the existence of unique photoreceptor in skin and its role in photoimmunology. *J Exp Med* 157:84-98(1983).
 26. Vos JG, Ruitenberg EJ, Van Basten N, Buys J, Elgersma A, Kruizinga W. The athymic nude rat. IV. Immunocytochemical study to detect T cells, and immunological reactions against *Trichinella spiralis*. *Parasite Immunol* 5:195-215(1983).
 27. Ruitenberg EJ, Elgersma A. Absence of intestinal mast cell response in congenitally athymic mice during *Trichinella spiralis*. *Nature* 264:250-260(1976).
 28. Daynes CK, Spellman CW. Evidence for the generation of suppressor cells by ultraviolet radiation. *Cell Immunol* 31:182-187(1977).
 29. Goettsch W, Garssen J, De Gruijl FR, Van Loveren H. UV-B and the immune system. A review with special emphasis on T cell-mediated immunity. *Thymus* 21:93-114(1993).
 30. Simon JC, Cruz PD, Bergstresser PR, Tigelaar RH. Low dose ultraviolet B-irradiated Langerhans cells preferentially activate CD4+ cells of the T helper 2 subset. *J Immunol* 145:2087-2091(1990).
 31. Simon JC, Tigelaar RH, Bergstresser PR, Edelbaum D, Cruz PD. Ultraviolet B radiation converts Langerhans cells from immunogenic to tolerogenic antigen-presenting cells. Induction of specific clonal anergy in CD4+ helper 1 cells. *J Immunol* 146:485-491(1991).
 32. Stahlmann R, Chahoud I, Korte M, Thiel R, Van Loveren H, Vos JG, Forster M, Merker HJ, Neubart D. Structural anomalies of thymus and other organs and impaired resistance to *Trichinella spiralis* infection in rats after prenatal exposure to acyclovir. *Thymus Update* 4:129-155(1991).
 33. Vos JG, De Klerk A, Krajnc EI, Kruizinga W, Van Ommen B, Rozing J. Toxicity of bis(tri-n-butyltin)oxide in the rat. II. Suppression of the thymus-dependent immune responses and of parameters of non-specific resistance after short-term exposure. *Toxicol Appl Pharmacol* 75:387-408(1984).
 34. Vos JG, Van Loveren H. Assessment of effects of dietary exposure to toxic compounds on local immunity in the rat. *Toxicologist* 8:11(1988).
 35. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 136:2348-2357(1986).
 36. Cox FEG, Liew FY. T cell subsets and cytokines in parasitic infections. *Immunol Today* 13:445-448(1992).
 37. Rivas JM, Ullrich SE. Systemic suppression of delayed-type hypersensitivity by supernatants from UV-irradiated keratinocytes. An essential role for keratinocyte-derived IL-10. *J Immunol* 149:3865-3871(1992).
 38. Sontag Y, Garssen J, De Gruijl FR, Van der Leun JC, Van Vloten WA, Van Loveren H. UVB induces suppression of the early initiating phase of contact hypersensitivity to picrylchloride. *Photochem Photobiol* 53:245(1991).

Volume 101, Supplement 6, December 1993

Environmental Health
perspectives
Supplements



Health Effects of Gasoline

Volume 101, Supplement 6, contains the proceedings of the Symposium on the Health Effects of Gasoline held November 5-8, 1991, in Miami, Florida. The main objective of the Symposium was to determine what evidence exists for the adverse health effects of gasoline. Sponsors of the conference were the petroleum and automotive industries, government agencies, and private research institutions from the United States, England, Japan, and Sweden.

To order your copy, contact:
Government Printing Office
Washington, DC 20402
or call 202-512-2406.