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Addendum to the Final Report on the Safety Assessment of Hydroquinone

Summary: An assessment of the safety of Hydroquinone was first published in 1986 (*J Am Coll Toxicol* 5:123–65). The ingredient was found to be safe for use at limited concentrations for certain formulations. This addendum reviews new data and presents a revised conclusion regarding safety. Hydroquinone is an aromatic compound used principally in hair dyes and colors, but it is also in lipsticks, skin fresheners, and other skin care preparations. Hydroquinone in an aqueous solution was shown to be absorbed through human skin at a rate of 0.55 ± 0.13 $\mu g/cm^2/h$. Hydroquinone is rapidly absorbed and excreted in urine in rats following oral administration. Absorption from an alcohol vehicle is greater than from an aqueous solution. Hydroquinone was found to be cytotoxic to rat hepatoma cells in culture, and nephrotoxic in male rats dosed orally by gavage. Oral administration of Hydroquinone to rats resulted in dose-dependent mortality, lethargy, tremors, and increased liver and kidney weights. Oral administration did not produce embryotoxic, fetotoxic, or teratogenic effects in rats. In rats, dermal application produced slight to severe irritation. In a guinea pig maximization test, induction with 2% Hydroquinone injected intradermally, followed by challenge with 0.5% Hydroquinone, showed extreme sensitization. In 80 patients known to be sensitive to aromatic compounds, 0.5% Hydroquinone elicited no reactions. Hydroquinone can cause depigmentation of skin. Various genotoxicity assays show that Hydroquinone can induce sister chromatid exchanges, chromosomal aberrations and loss, and increased frequency of mitotic crossovers. It also induced DNA strand breaks and inhibited DNA and RNA synthesis in rabbit bone marrow mitochondria. Forward mutation assays with or without metabolic activation were positive, but the results with the Ames test, a mouse test for somatic mutations, and other tests were negative. Hydroquinone, given to rats orally by gavage five times per week for up to 103 weeks at doses of 25 or 50 mg/kg, resulted in a significant increase of renal adenomas in males given 50 mg/kg and of mononuclear cell leukemia in females with both doses. At doses of 50 or 100 mg/kg on the same schedule, there was a significant increase in hepatocellular adenomas in both male and female mice. Other studies of Hydroquinone showed no significant difference in tumors between control and exposed groups, and marginal to no activity as a tumor promoter. It is concluded that Hydroquinone is safe at concentrations of $\leq 1\%$ for *aqueous* cosmetic formulations designed for discontinuous, brief use followed by rinsing from the skin and hair. Hydroquinone should not be used in leave-on, nondrug cosmetic products. **Key Words:** Safety assessment—Hydroquinone.

The Cosmetic Ingredient Review (CIR) Expert Panel has previously concluded that Hydroquinone (CAS No. 123-31-9) is safe for cosmetic use at $\leq 1\%$ in formulations designed for discontinuous, brief use followed by rinsing from the skin and hair (Elder, 1986). The carcinogenicity data used to reach the original con-

TABLE 1. *Product formulation data for Hydroquinone (FDA, 1993)*[a]

Product category	Total no. of formulations in category	Total no. containing ingredient
Hair dyes and colors	1,112	185
Lipstick	937	2
Skin fresheners	246	1
Other skin care preparations	848	18
1993 Totals		206

[a] CIR requests that the cosmetic industry provide current formulation data on each product category.

clusion were primarily from a dermal study of a complete hair dye formulation that contained 0.2% Hydroquinone. In 1989, the National Toxicology Program (NTP) issued a report on a carcinogenicity study in which Hydroquinone was administered orally by gavage. This literature update includes the results of that study and other relevant data that have been published since 1983 and not cited in the previous safety evaluation.

The safety of Hydroquinone was reviewed primarily for its use in hair dyes, not for other uses, as demonstrated in the following excerpt from the Discussion of the original report:

> ". . . members of the Expert Panel were aware that the Food and Drug Administration had reviewed studies relating to the safety of Hydroquinone and had concluded that Hydroquinone was safe and effective as an agent to bleach or lighten the skin at concentrations between 1.5 and 2.0% in OTC drugs. The CIR Expert Panel has not evaluated the safety of Hydroquinone to lighten the skin, which is regarded by FDA as a drug use of this ingredient."

This report deals with the use of Hydroquinone in cosmetic leave-on preparations.

Hydroquinone is an aromatic compound (Nikitakis, 1988) that is used in hair dyes and colors, lipsticks, skin fresheners, and other skin care preparations (FDA, 1993). In 1981, it was reported to the Food and Drug Administration (FDA) that Hydroquinone was used in 170 cosmetic ingredient formulations, including 147 hair dye formulations (FDA, 1981). The number of cosmetic formulations in which Hydroquinone is used has increased since 1981; in 1993, it was reported to the FDA that Hydroquinone was used in 206 formulations, 185 of which were hair dye formulations (Table 1).

Concentration of use values are no longer reported to the FDA by the cosmetic industry (Federal Register, 1992). However, product formulation data submitted to the FDA in 1984 indicated that Hydroquinone was used at concentrations of $\leq$1% in hair dyes and colors requiring caution statements and at 1–5% in skin cleansing products and other skin care preparations (FDA, 1984).

CHEMISTRY

Analytical Methods

The purity of Hydroquinone, derivatized and underivatized samples, has been determined using gas chromatography with a flame ionization detector (GC/FID)

(Eastman Kodak Company, 1988, 1989). The structure of Hydroquinone was confirmed by gas chromatography–mass spectrometry (GC/MS), using electron impact ionization (EI).

Rate of Hydroquinone Disappearance During Its Use in Hair Dyes

Hydroquinone, which acts as a regulating agent allowing some control of the color-forming coupling reactions, is a "consumable" in the hair dyeing procedure, with its actual concentration decreasing as the color-forming reactions proceed (L'Oreal, 1990).

To determine how much of the Hydroquinone is consumed, three commercial hair dye formulations were used to measure the rate of disappearance of Hydroquinone during the hair dyeing procedure (L'Oreal, 1990). Two of the formulations contained 0.08% (w/w) and the third contained 0.15% (w/w) Hydroquinone. An oxidation cream containing 6% (w/w) hydrogen peroxide was used for oxidation of the mixtures.

The test article was prepared by mixing 15 g of the cream oxidant with 15 g of the hair dye. It was then uniformly applied with a paintbrush to a 30 cm long (15 g) lock of natural hair. After 5, 10, 20, and 30 min, 2.5 g samples of the hair dye mixture were collected from the hair lock and assayed in a high-performance liquid chromatography (HPLC) system. (Results of the system calibration showed that it responded linearly with respect to concentration of Hydroquinone for the range 0.8–80 μg/ml.)

For each of the three formulations, the percentage of residual Hydroquinone was determined as a function of time. The amount of Hydroquinone that remained in the mixture decreased sharply as the oxidation reaction proceeded. Within 4 min, 50% of the Hydroquinone was already consumed. After 30 min, the residual Hydroquinone was <3% of the initial amount, regardless of which formulation was used. During the initial 20 min, the amount of Hydroquinone remaining varied for the two formulations containing 0.08%. However, the difference in the amount remaining for these two formulations decreased after 20 min.

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, Excretion

Hydroquinone occurs as 4-hydroxyphenyl-β-D-glucopyranoside (arbutin) in several types of plants, including cranberries, blueberries, and pears, and it is likely that Hydroquinone is found in other sources in the diet, such as coffee and whole wheat bread (Hill et al., 1993). Consumption of a diet containing foods with a higher natural Hydroquinone concentration leads to a greater concentration of Hydroquinone in the urine.

The in vitro rate of percutaneous absorption of an ~5% aqueous solution of [^{14}C]Hydroquinone was determined for humans using stratum corneum and for rats using full thickness skin (Barber et al., 1992). Five samples were used for each. The rate of absorption was 0.522 ± 0.13 μg/cm^2/h for human stratum corneum and 1.09 ± 0.65 μg/cm^2/h for rat skin. The Hydroquinone solution produced

little or no damage to the skin samples, with "damage ratios" of near 1 for both the human and rat skin. The permeability constant (K_p) for Hydroquinone through human stratum corneum was 9.58×10^{-6} cm/h. Using the terminology of Marzulli et al. (1969), Hydroquinone would be classified as a "slow" permeant for human skin.

Preparations containing [^{14}C]Hydroquinone and varying amounts of a penetration enhancer, 1-dodecylazacycloheptan-2-one (Azone), and a sunscreen, the 2-ethylhexyl ester of 4-(dimethylamino)benzoic acid (Escalol 507), were prepared in an alcoholic vehicle and used to determine the percutaneous absorption of Hydroquinone in humans (Bucks et al., 1988). Each solution contained 2% (w/w) Hydroquinone, 26.8% (w/w) water, 0.2% (w/w) ascorbic acid, and 67.5–71.0% (w/w) ethanol (95%). Solutions 1 and 3 contained 3.0% (w/w) Escalol 507 and solutions 1 and 2 contained 0.5% (w/w) Azone. (Solution 4 contained neither the sunscreen nor the enhancer.) Six male subjects were used per group. A single 100-μl dose of each [^{14}C]Hydroquinone solution (4.8 μCi) was applied to a 16-cm^2 area of skin on the forehead (125 μg/cm^2) for 24 h. The subjects collected all their urine for the intervals 0–4, 4–8, 8–12, and 12–24 h and days 1–5 following application.

The average percutaneous absorption of Hydroquinone was estimated from the amount eliminated in the urine. Hydroquinone was readily absorbed through the skin from an alcoholic vehicle, with estimated absorption of 35 ± 17% for solution 1, 66 ± 13% for solution 2, 26 ± 14% for solution 3, and 57 ± 11% for solution 4. Peak elimination occurred within the first 12 h following application, with total elimination occurring within 5 days. Escalol 507 significantly inhibited the absorption of Hydroquinone while Azone did not significantly affect absorption.

Six fasted male beagle dogs were used to determine the dermal absorption of [U-^{14}C]Hydroquinone (Eastman Kodak Company, 1985*a*). Approximately 40 μCi, 15 ml, of a 4.5 g/L Hydroquinone in saline solution was added to a sealed glass absorption cell that was attached to the shaved thorax of each dog. (Cotton gauze was placed inside the cell for uniform distribution of the solution.) A 55.6-cm^2 area of skin was exposed to Hydroquinone for 60 min and then washed and rinsed. Blood samples and urine were collected at intervals for 48 and 120 h, respectively. Radioactivity was then measured by liquid scintillation counting.

The urinary excretion of radioactivity was low, with the greatest concentrations being detected in the 24- and 48-h collections. The total amount of ^{14}C excreted after 48 and 120 h was equivalent to ~149 and 170 nmol (11.9 and 13.6 μg) of Hydroquinone, respectively. A measurable concentration of [^{14}C]Hydroquinone (<0.025 μg/ml) was not detected in the blood after 60 min dermal exposure.

Fasted male beagle dogs were used to determine the distribution of [U-^{14}C]Hydroquinone after intravenous (i.v.) administration (Eastman Kodak Company, 1985*a*). Approximately 3 or 4 ml of a [^{14}C]Hydroquinone solution was administered rapidly through an indwelling foreleg i.v. catheter at doses of 1 mg/kg (10 or 100 μCi) and 10 mg/kg (30 μCi) to eight and three dogs, respectively. Expired air was collected continuously for 8 h from the low-dose group. After dosing, urine samples were collected at 4, 8, and 12 h and blood samples were collected at 8 and

12 h from both groups. Urine, feces, and blood samples were collected daily for 6 or 7 days. Major organs and tissues were excised from two dogs and assayed for residual radioactivity by liquid scintillation counting.

The radioactivity in the blood comprised 7–8% of the dose after 4 h for both groups; this value decreased to ~1% by 24 h. Blood ^{14}C concentrations declined slowly from 19.0 nmol Eq/ml at 5 min to 1.1 nmol Eq/ml by 24 h following the 1 mg/kg dose and from 133.2 nmol Eq/ml at 5 min to 11.4 nmol Eq/ml by 24 h following the 10 mg/kg dose. There was an apparent absorption, distribution, and elimination phase during the first 24 h. The estimated mean half-life values for the α and β phases were 1.3 and 7.2 h, respectively, for 1 mg/kg Hydroquinone and 1.0 and 8.0 h, respectively, for 10 mg/kg Hydroquinone.

Urinary excretion was rapid initially, with the 8- and 24-h cumulative excretion totals being 20 and 26%, respectively, for the 1 mg/kg dose and 53 and 60%, respectively, for the 10 mg/kg dose. The majority of the radioactivity was eliminated within 4 h following dosing. Between days 2 and 5, only an additional 6–7% of the radioactivity was excreted at either of the doses. At a dose of 1 mg/kg Hydroquinone, 34.5% of the radioactivity was accounted for by urinary excretion after 7 days. For the 10 mg/kg dose, 65.7% of the radioactivity was accounted for by urinary excretion after 5 days.

After 48 h, 4.6 and 2.5% of the radioactivity was recovered in the feces of the 1 and 10 mg/kg dose group animals, respectively. After 4–5 days, these values were 7.5 and 6.1%, respectively. The amount of radioactivity in the expired air of animals of the 1 mg/kg dose group was below the limit of detection (<0.05% of the dose). The overall recovery of ^{14}C at 4–7 days was 42.0 and 71.8% for the low- and high-dose groups, respectively.

Twenty-four hours after i.v. administration of 1 mg/kg [^{14}C]Hydroquinone, the skin contained an equivalent of 10.4% of the dose, the liver 0.6%, and the intestine 0.5%.

Using the 48-h excretion data from both the previous dermal and i.v. absorption studies, the average percutaneous absorption rate was determined to be ~0.15 nmol/cm^2/min (1.1 μg/cm^2/h) (Eastman Kodak Company, 1985*a*).

Fasted female Fischer 344 rats, five per group, were given a single oral dose of 5, 25, or 50 mg/kg [U-^{14}C]Hydroquinone (10 μCi) in degassed, distilled water by gavage (Eastman Kodak Company, 1985*b*). Six rats, two per dose group, were given vehicle only and served as controls. Urine, feces, and expired CO_2 were collected for 24 h. At the end of this period, all animals were killed and various tissues were analyzed for radioactivity.

[U-^{14}C]Hydroquinone was rapidly absorbed and excreted. At all concentrations, $\geq$86.7% of the dose was excreted in the urine within the first 8 h after administration and $\geq$91.8% was excreted within 24 h of administration. After 8 h, 0.3–1.9% of the dose was recovered in the feces and after 24 h, 2.3–2.9% of the dose was recovered in the feces. Approximately 0.26–0.36% of the dose was recovered as expired CO_2 after 24 h.

Upon examination of selected tissues, the liver had the greatest concentration of radioactivity and contained 0.51–0.62% of the dose. The intestine and kidneys contained $\leq$0.06% of the dose, whereas other examined tissues contained $\leq$0.02%

of the dose. Between 0.64 and 0.90% of the dose was recovered in the carcass. A total of 1.26–1.64% of the dose was found in the examined tissues and carcass. The radioactivity was extractable in the small intestine (20–39%), carcass (14–24%), liver (1.7–3.7%), and kidneys (0–2.4%).

After 24 h, the overall recovery was ≥96.1%. Elimination of Hydroquinone was not dose-related for female Fischer 344 rats. Very little of the dose was excreted as unchanged Hydroquinone. After 8 h, Hydroquinone glucuronide accounted for 43.4–49.0% of the dose in the urine, and Hydroquinone sulfate accounted for 28.8–35.9%. Only 2.1–3.0% of the dose was excreted as unchanged Hydroquinone. Other metabolites of Hydroquinone were not detected.

Lightly anesthetized fasted male Fischer 344 rats, five per group, were given a single dose of 5, 25, or 50 mg/kg [U-^{14}C]Hydroquinone (10 μCi) in degassed, distilled water by intratracheal instillation (Eastman Kodak Company, 1985*c*). Six rats, two per dose group, were given vehicle only and served as controls. Urine, feces, and expired CO_2 were collected for 48 h. At the end of this period, all animals were killed and various tissues were analyzed for radioactivity.

As with the female rats in the previous study, [U-^{14}C]Hydroquinone was rapidly absorbed and excreted by male Fischer 344 rats. At all concentrations, ≥80.74% of the dose was excreted in the urine within 8 h of administration and ≥91.68% was excreted within 48 h of administration. In the feces, 0.56–1.5% of the dose was recovered after 8 h; this value was 1.3–2.84% after 48 h. Approximately 0.13–0.20% of the dose was recovered as expired CO_2 in 48 h. A total of ≥93.86% of the dose was excreted in the urine, feces, and expired air after 48 h.

Upon examination of selected tissues, the liver contained 0.12–0.43% of the dose, the lungs contained ≤0.13%, the kidneys contained ≤0.08%, the intestine, brain, testes, and fat contained ≤0.04%, and the femur, heart, and spleen each contained <0.01% of the dose. Between 0.93–1.71% of the dose was recovered in the carcass, and a total of 1.33–2.46% of the dose was found in the examined tissues and carcass. Significant extractable radioactivity was found in the lungs and the carcass.

After 48 h, the overall recovery was ≥96.04%. Urinary conjugates were the major metabolites of Hydroquinone. Hydroquinone glucuronide accounted for 48.76–67.21% of the dose in the urine, and Hydroquinone sulfate (ethereal) accounted for 19.00–22.07%. Only 2.00–2.85% of the dose was excreted as unchanged Hydroquinone.

A two-phase study was conducted using fasted male Fischer 344 rats to determine the effect of the route of administration of 50 mg/kg [U-^{14}C]Hydroquinone (10 μCi) on blood elimination kinetics (Eastman Kodak Company, 1986). Prior to Phase I, a one-piece jugular catheter was implanted in each rat using a modification of the procedure by Upton (1975). In Phase I, lightly anesthetized rats, 3–4 per group, were given a single dose of Hydroquinone by gavage, intratracheal (i.t.) administration, or femoral i.v. injection. (For the rats receiving an i.v. injection, a second catheter was implanted in the femoral vein 2 days after implantation of the first catheter.) Blood samples were collected at various times for up to 8 h after dosing. At the end of this period, the animals were killed and their lungs removed.

In Phase II, three rats per collection period were dosed with Hydroquinone by gavage. At 10 min, 20 min, 40 min, 1 h, 2 h, or 4 h after dosing, blood was obtained from anesthetized rats via cardiac puncture and the rats were then killed.

In Phase I, >80% of the dose was excreted in the urine. For all routes of administration, distribution equilibrium was attained after 1.5–2.5 h. After i.v. administration of [^{14}C]Hydroquinone, the blood concentration time curves of total radioactivity were biphasic, with mean half-lives of 18.7 and 326 min. After administration by gavage, the average absorption rate constant was 1.3 min, the T_{max} for radioactivity in the blood was 6.5–7.5 min, and the decline of blood radioactivity was biphasic, with average half-lives of 14.8 and 626 min. Following i.t. administration, radioactivity absorption into the blood was very rapid; the maximum concentrations of radioactivity were obtained by the first sampling period at 2 min. The blood concentration time curves for i.t. administration were triphasic, with mean half-lives of 1.1, 22.1, and 425 min.

In Phase II, the mean concentrations of ^{14}C in the whole blood, plasma, plasma ultrafiltrates, and blood cells were determined. During 0–4 h, the concentration of ^{14}C in the blood, plasma, and plasma ultrafiltrates decreased. In blood cells, the radioactivity concentration declined during the first hour, and then remained constant. At this time, ~64% of the radioactivity in the blood was associated with the cells. At 4 h, the ^{14}C concentration in the plasma was greater than twofold the amount in the plasma ultrafiltrate.

In the plasma filtrate from the animals treated with [^{14}C]Hydroquinone, at least four electrochemically active compounds were present that were not present in the plasma ultrafiltrate from untreated animals. Free Hydroquinone represented <1% of the total radioactivity in the plasma ultrafiltrate, declined rapidly during the first hour, and remained below the limit of detection (viz., 10 ng/g plasma ultrafiltrate) through 4 h. The conjugates of Hydroquinone, predominantly Hydroquinone glucuronide, were present in the plasma ultrafiltrate 40 min after administration of [^{14}C]Hydroquinone by gavage.

Fischer 344 rats were used to determine the metabolic fate of Hydroquinone following a single oral dose, multiple oral doses, or dermal application (Eastman Kodak Company, 1988*b*). Each group consisted of 16 rats, eight per sex.

In the single oral dose study, two groups of rats were dosed with either 25 or 350 mg/kg [^{14}C]Hydroquinone (10 μCi) by gavage. For the multiple oral dose study, rats were dosed with 25 mg/kg nonradioactive Hydroquinone for 14 days and 25 mg/kg [^{14}C]Hydroquinone (10 μCi) on day 15 by gavage. The animals were fasted 4 h after dosing. For the dermal application, annular columnators were secured to the shaved dorsal surfaces of the rats, providing an exposure area of 2 cm^2. Two groups of rats were then given applications of 25 mg/kg (58–86 μl) or 150 mg/kg (338–497 μl) [^{14}C]Hydroquinone (20 μCi); the application site was covered by an occlusive patch for 24 h and then rinsed. (Technical difficulties were encountered in containing the Hydroquinone solutions to the 2 cm^2 application site.)

In each group, eight rats, four per sex, were used for collection of excreta and the remaining eight were used for collection of blood samples. The studies were conducted for at least 48 h and were generally terminated when ~90% of the radioactivity was recovered in the excreta. Excreta, such as urine and feces, and

cage rinsings were collected at 8 and 24 h after dosing and then every 24 h for up to 7 days. Blood samples were obtained from the orbital sinus at intervals for up to 96 h. At study termination, the animals were killed and various tissues were analyzed for radioactivity.

Radioactivity was rapidly excreted in the urine following oral administration of [^{14}C]Hydroquinone. There was no significant difference observed between males and females. In the single oral dose study using 25 mg/kg Hydroquinone, ~81–82% and 89% of the dose was recovered in the urine after 8 and 24 h, respectively. In the group dosed with 350 mg/kg Hydroquinone, urinary radioactivity excretion after 8 h was 54 and 45% of the dose by males and females, respectively, and 85 and 82% of the dose, respectively, after 24 h. Following repeated dosing with 25 mg/kg, males and females excreted 77 and 87% of the dose in the urine, respectively, after 8 h, and 91 and 93% of the dose, respectively, after 24 h. Approximately 1–3% of the radioactivity was recovered in the feces after 24 h. (For one low-dose female, the urine and feces were not well separated and artifactually high radioactivity values were reported in the feces.)

Due to the difficulty in containing the Hydroquinone solution to the test site during dermal administration, the amount of Hydroquinone recovered by rinsing varied greatly after 24 h, from 23.1 to 98.5% of the radioactivity, regardless of sex. After dermal application of either 25 or 150 mg/kg Hydroquinone, mean values of the recovered radioactivity ranged from 61 to 71% following rinsing at 24 h. The mean total recovery of ^{14}C in the urine ranged from 7.8 to 12.8%, and was recovered primarily in the initial 48 h. The mean 7-day ^{14}C recovery in the feces was 1.7–3.7% of the dose, and the mean value recovered in the chamber rinsings ranged from 3.8 to 8.9%. (Due to application difficulty, the recovered radioactivity in the urine, feces, and chamber rinsings may not have been solely from the intended application site.)

The mean amount of radioactivity recovered in the tissues and carcass was 0.51–0.96% of the dose following oral administration. The liver and the kidneys had the greatest radioactivity concentrations, with greater concentrations being recovered from females than males. After all doses, ~0.2% of the dose was recovered from the livers of female rats. For males, 0.075% of the dose was recovered from the liver after single and repeated dosing with 25 mg/kg Hydroquinone, and 0.24% was recovered from the liver following a single administration of 350 mg/kg Hydroquinone. Approximately 0.02 and 0.01% of the dose was recovered from the kidneys of female and male rats, respectively, after single and repeated doses of 25 mg/kg, whereas 0.04 and 0.03% of the dose was recovered from the kidneys of the female and male high-dose group rats, respectively. The ^{14}C concentrations in the other examined tissues were not significantly different from the concentration in the carcass.

The mean radioactivity recovered from the application site ranged from 0.1 to 2.2% of the dose, and 2.6–12.9% of the dose was recovered from the carcass and other tissues. The percentage of ^{14}C recovered from the application site and from other tissues was highly variable following dermal application. The total ^{14}C recovered after dermal administration was 90.1–94.6% of the dose.

After oral administration of [^{14}C]Hydroquinone, the blood radioactivity con-

centration declined biphasically and was near or below the limits of quantitation (~0.03% of the dose) 24 h after dosing. After 8 h, the mean blood concentrations ranged from 0.1 to 0.4% of the dose. After a single oral dose of 25 mg/kg Hydroquinone, peak blood concentrations were 7.62 μg Eq/g blood for males, reached at 0.24 h, and 7.19 μg Eq/g blood for females, reached at 0.32 h. Following a single oral dose of 350 mg/kg Hydroquinone, peak blood concentrations were 39.2 μg Eq/g blood for males, reached at 0.56 h, and 45.8 μg Eq/g blood for females, reached at 0.80 h. After repeated oral doses of 25 mg/kg Hydroquinone, peak blood concentrations were 6.61 μg Eq/g blood for males, reached at 0.16 h, and 6.03 μg Eq/g blood for females, reached at 0.24 h. The half-life of the α phase (α $T_{1/2}$) ranged from 0.2 to 1.7 h for males and females, with the greater values being observed for the high-dose group. Estimates of the half-life of the β phase (β $T_{1/2}$) varied and could not be determined accurately because of the appearance of a second peak in the blood concentration versus time curve; the range was 2.8–10.5 h.

With the exception of some female animals, blood ^{14}C concentrations were below the limit of quantitation (~0.01% of the dose or 0.06 μg Eq Hydroquinone/g blood at the low dose) following dermal application of Hydroquinone. The greatest individual values observed were 0.47 μg Eq Hydroquinone/g blood at 0.5 h for the low-dose group and 1.13 μg Eq Hydroquinone/g blood at 1.0 h for the high-dose group. Blood ^{14}C concentrations were not detectable at later collection times.

The amount of unchanged Hydroquinone in the urine ranged from undetectable (~0.05% of the dose) to 7.12% of the dose. Hydroquinone glucuronide accounted for 45–53% of the dose whereas Hydroquinone sulfate accounted for 19–33%. A small amount of the dose was excreted in the urine as the mercapturic acid conjugate of Hydroquinone.

The systemic bioavailability of dermally relative to orally administered Hydroquinone was estimated by the ratio of the urinary excretion of radioactivity after dermal and oral administration of 25 mg/kg [^{14}C]Hydroquinone. The percentage of dermal absorption in male rats was estimated to be 10.5% using 72-h urine ^{14}C recovery data. Using cumulative 48-h ^{14}C recovery data, the percentage of dermal absorption in female rats was estimated to be 11.5%.

IMMUNOLOGICAL EFFECTS

Hydroquinone is a hepatic metabolite of benzene that is carried by the blood to the bone marrow (Thomas et al., 1989); following benzene administration, high persistent concentrations of Hydroquinone and other benzene metabolites have been found in the bone marrow (Andrews et al., 1987; Rickert et al., 1979). These metabolites may accumulate in bone marrow and adversely affect hematopoiesis (Brown et al., 1987).

Two suggested mechanisms of benzene toxicity are: (a) the direct action of benzene metabolites, including Hydroquinone, with hematopoietic cells, and (b) by the action of benzene or its metabolites on bone marrow stromal cells (Gaido and Wierda, 1987). The combined impact of benzene metabolites on specific

cellular sites may be the cause of failure of bone marrow cellular proliferation after treatment with benzene (Snyder et al., 1989).

It should also be noted that Irons et al. (1992) has stated that "the potential of Hydroquinone to alter intrinsic growth factor response and induce differentiation in a myeloid progenitor cell population may be important to the pathogenesis of acute myelogenous leukemia secondary to benzene exposure. Benzene leukemogenesis may result from the dual ability of its metabolites to promote progenitor cell differentiation and induce cytogenetic changes in replicating cells."

Additional work has shown that the metabolism by pathways leading to the Hydroquinone conjugates of benzene in the cynomolgus monkey, as in rats and mice, was saturated at relatively low doses of benzene (Sabourin et al., 1992). The profile of urinary metabolites of benzene produced by the cynomolgus monkey was qualitatively similar, but quantitatively different, from that observed using rodents. The researchers believe that, since this dose effect has been observed in three different species, it is reasonable to expect that the same enzymatic pathways will also have a limited capacity in humans. The researchers stated: "If one assumes that urinary levels of Hydroquinone conjugates [and muconic acid] parallel tissue levels of Hydroquinone [and muconaldehyde], and that Hydroquinone [and muconic acid], either alone or in combination with other phenolic metabolites, are involved in benzene toxicity, then the saturation of pathways leading to Hydroquinone [and muconic acid] at low doses must be considered in any risk assessment."

Many of the studies summarized in this section also investigated the effects of benzene, phenol (a metabolite of benzene), and other metabolites of benzene, but only the results of exposure to Hydroquinone or Hydroquinone and phenol have been included.

C57BL/6 mice, four per group, were dosed either by i.v. or intraperitoneal (i.p.) injection twice daily for 3 days with 100 mg/kg Hydroquinone (Irons et al., 1983). Hydroquinone was immunosuppressive; spleen and especially bone marrow cellularity was reduced. A suppression of lipopolysaccharide (LPS) and LPS plus dextran sulfate (DXS)–stimulated plaque-forming cells was observed.

Groups of six male B6C3F1 mice were given i.p. injections, twice daily at 6-h intervals, of Hydroquinone and Hydroquinone with phenol (Eastmond et al., 1987). Hydroquinone administration (100 mg/kg, 2 times/day) produced a transient mild suppression of bone marrow cellularity after 3 days; after 12 and 36 days of dosing, bone marrow cellularity of the test group was not significantly different from that of the controls. Coadministration of 25–75 mg/kg Hydroquinone and 75 mg/kg phenol produced a significant decrease in cellularity after 12 days of dosing; a dose–response relationship was evident.

Two groups of five male DBA/2 mice were dosed concomitantly with 50 mg/kg Hydroquinone in Dulbecco's phosphate-buffered saline (PBS) without calcium or magnesium (PBS A) (5 ml/kg) and 50 mg/kg phenol in corn oil (5 ml/kg) by i.p. injection twice daily for 2 days (Pirozzi et al., 1989). A group of five male DBA/2 mice was dosed with 2 mg/kg indomethacin, an inhibitor of the cyclooxygenase component of prostaglandin H synthase (PHS), 1 h before and 3 and 6 h after being dosed with Hydroquinone/phenol. Two groups of five mice were dosed with

4 or 8 mg/kg of meclofenamate, also an inhibitor of the cyclooxygenase component of PHS, 1 h before dosing with Hydroquinone/phenol. Two control groups were dosed with vehicle, one group was given vehicle consisting of corn oil and 4.2% ethanol in PBS A and the other vehicle consisted of corn oil, water, and PBS A. The mice were killed and their femurs removed 17 h after the last dose. Administration of Hydroquinone/phenol significantly depressed bone marrow cellularity when compared to the controls. Neither indomethacin nor meclofenamate prevented the marrow depression caused by Hydroquinone/phenol.

Groups of DBA/2 mice, six to eight mice per group, were given a single i.p. injection of 50 mg/kg Hydroquinone in PBS A and 50 mg/kg phenol in corn oil, Hydroquinone/phenol and 8 mg/kg indomethacin, or vehicle (Pirozzi et al., 1989). The indomethacin was injected 1 h before dosing with Hydroquinone/phenol. The mice were killed and their femurs removed 4–5 h after dosing. Hydroquinone/phenol significantly increased bone marrow prostaglandin E (PGE) concentration when compared with controls; indomethacin prevented this effect.

Male B6C3F1 mice were given an i.p. injection of 75 mg/kg [^{14}C]Hydroquinone (10 μCi/25 g body wt) and killed 4 or 18 h after dose administration, with liver, kidneys, blood, and bone marrow samples being analyzed for covalent binding of radioactivity to acid-insoluble macromolecules (Subrahmanyam et al., 1990). The binding of [^{14}C]Hydroquinone metabolites to hepatic, bone marrow, and renal macromolecules, but not blood macromolecules, was significantly greater after 18 h compared with the values for 4 h. At 18 h, the amount of radioactivity covalently bound in the liver was 2-, 8-, and 10-fold greater than that in the kidneys, blood, and bone marrow, respectively.

In mice given two injections of 75 mg/kg [^{14}C]Hydroquinone, 6 h apart, and then killed 18 h after the second dose, a significant difference in ^{14}C associated with tissue macromolecules was not observed. This may have been due to dilution of the radioactivity.

Also in the study by Subrahmanyam et al. (1990), Hydroquinone and phenol were coadministered (doses not given). The amount of [^{14}C]Hydroquinone oxidation products covalently bound to blood and bone marrow macromolecules was significantly increased with Hydroquinone and phenol coadministration as compared with the administration of Hydroquinone alone. No significant change was observed in the amount of oxidation products bound in the liver and kidney.

Marrow cells obtained from the femurs of male B6C3F1 mice were treated with 10^{-7}–10^{-5} *M* Hydroquinone for 1 h and then functional B cells were assayed after 0, 24, 48, or 72 h incubation (King et al., 1987). Control cultures were also grown. Bone marrow cell cultures were manipulated and unmanipulated, that is, depleted of sIgM$^+$ cells and not depleted of sIgM$^+$ cells, respectively.

In the manipulated cell cultures, decreases in the number of pre-B cells in culture were delayed for at least 48 h by Hydroquinone administration when test cultures were compared with the controls; the number of small pre-B cells was significantly increased after treatment with all doses of Hydroquinone. The number of large pre-B cells was not significantly affected by Hydroquinone.

Twenty-four hours after treatment with Hydroquinone, the number of sIgM$^+$

cells was significantly less than the control value. The same pattern of altered B-lymphopoiesis was also seen in unmanipulated bone marrow cell cultures.

After treatment with Hydroquinone, marrow-adherent cell clusters (>25 cells) were counted and the number of adherent cell clusters was less than the control value after 48 and 72 h. Hydroquinone caused a concentration-dependent decrease in B-lymphocyte colony formation in culture (BL-CFC) after 24 and 48 h when the unmanipulated cultures were compared with the controls; similar results were observed for B cell–depleted cultures after treatment with Hydroquinone. The authors stated that phenotypic analysis of pre-B cell maturation in liquid bone marrow cultures suggested that this may be due to an inability of pre-B cells to mature into $sIgM^+$ B cells.

After 72 h, BL-CFC frequency of Hydroquinone-treated cultures was similar to control values. LPS-stimulated B cell proliferation in unmanipulated cultures was not affected by Hydroquinone treatment. The number of adherent stromal cell colonies that develop in short-term liquid bone marrow cultures was reduced by Hydroquinone; this may have been the reason for Hydroquinone-induced inhibition of pre-B cell maturation.

The authors noted that 10^{-7} *M* Hydroquinone, the lowest concentration tested in this study, inhibited pre-B cell maturation; theoretically, this is the same concentration of Hydroquinone attained by humans exposed to 10 ppm benzene for 8 h (the current Occupational Health and Safety Administration threshold limit value) in the work place. The results of preliminary studies suggested that inhibition of pre-B cell maturation by Hydroquinone may be due to alterations in their interactions with adherent accessory cells. In this study, Hydroquinone exposure was acute; chronic exposure, which would be closer to actual environmental exposure, might even inhibit B-cell generation further (King et al., 1987).

The effect of in vitro Hydroquinone pretreatment on the intrinsic colony-forming response of murine bone marrow cells from male C57BL/6 mice stimulated with recombinant granulocyte/macrophage colony-stimulating factor (rGM-CSF) in the absence of conditioned medium was examined (Irons et al., 1992). Nonadherent bone marrow cells or enriched progenitor cells were pretreated with 10^{-8}–10^{-2} *M* Hydroquinone for 30 min. Colonies of ≥50 cells were scored on days 8 and 14. Phenol or catechol may have been coadministered with Hydroquinone.

Hydroquinone pretreatment of bone marrow cells resulted in a significant increase in GM-CSF–induced granulocyte/macrophage colonies (CFU-GM) at concentrations of 10^{-8}–10^{-5} *M*, with the maximum increase observed at 10^{-6} *M*. Concentrations of 10^{-3}–10^{-2} *M* suppressed CFU-GM. In the absence of GM-CSF, there was no colony formation with Hydroquinone pretreatment. Coadministration of phenol did not affect the extent of the Hydroquinone-induced increase in CFU-GM, but the optimal concentration–response was achieved at 10^{-10} *M*, not 10^{-6} *M*, Hydroquinone. Catechol coadministration prevented the increase in CFU-GM at 10^{-10}–10^{-6} *M* Hydroquinone and potentiated Hydroquinone-induced suppression by 100-fold in the absence of a demonstrable decrease in cell viability.

In the enriched progenitor cell population, bone marrow cells that were >96%

depleted of cells expressing lineage-specific markers, a twofold increase in GM-CSF–induced CFU-GM was obtained. Marginal increases in macrophage colony-forming units (CFU-M) obtained when bone marrow cells were pretreated with Hydroquinone were not seen with lineage-depleted cells. The authors stated that this suggested that "any effect of Hydroquinone on CFU-M either is mediated via a more differentiated responding cell population not present in lineage-depleted cells or requires accessory cell involvement."

Bone marrow cells from Swiss–Webster and C57B1/6J (C57) mice were incubated with 10, 20, or 40 μM Hydroquinone in NCTC-109 to assess bone marrow toxicity by determining the effect of Hydroquinone on the development of the colony forming unit-erythroid (CFU-e) (Neun et al., 1992). Cultures were also incubated with 10 μM Hydroquinone mixed with other benzene metabolites. Control cultures, which consisted of untreated bone marrow cells, were used for all experiments. Mean values of CFU-e colony formation were determined from six replicate cultures.

Hydroquinone produced differential toxicity to the CFU-e between the two strains of mice at 20 and 40 μM. Hydroquinone was more toxic to Swiss–Webster CFU-e than to C57 CFU-e. Combinations of Hydroquinone and other benzene metabolites affected the CFU-e of Swiss–Webster mice more severely than the CFU-e of C57 mice.

The hematopoietic toxicity of Hydroquinone was evaluated by determining the median inhibitory concentration (IC_{50}), the concentration for which the cell viability in treated cultures is 50% of the viability of untreated controls, for 48 h using a number of cell lines (Ruchaud et al., 1992). The IC_{50} of Hydroquinone at 48 h was 0.009 mM for the human promyelocytic leukemia NB4, 0.016 mM for the rat promyelocytic cell line IPC-81, 0.03 mM for the myelomonocytic cell line WEHI-3b D^+, and 0.014 mM for the murine factor–dependent cell line DA1.

Macrophage and bone marrow–derived fibroblastoid stromal cell line (LTF) cultures were exposed to 10 μM [^{14}C]Hydroquinone in serum-free medium for 24 h at 37°C to examine the role of selective bioactivation and/or deactivation on the macrophage-selective effects of Hydroquinone (Thomas et al., 1990). The amount of covalently bound, [^{14}C]Hydroquinone-derived radioactivity for macrophage cultures was 16-fold greater than that for LTF cells. A peroxide concentration-dependent bioactivation of Hydroquinone was observed in macrophage homogenates, but not in LTF cell homogenates, when increasing concentrations of hydrogen peroxide were added. In the absence of hydrogen peroxide there was no difference in bioactivation of Hydroquinone; with the addition of 40 μM hydrogen peroxide, maximal bioactivation in macrophage homogenates was fourfold greater than in LTF homogenates. Autoxidation resulted in binding amounts of >1 nmol [^{14}C]Hydroquinone Eq/mg protein for both macrophage and LTF cells.

The role of peroxidase in the bioactivation of Hydroquinone was examined. A time-dependent increase was observed in the amount of covalently bound radioactivity in samples containing hydrogen peroxide and myeloperoxidase (MPO); this increase was not seen in samples without hydrogen peroxide and/or MPO. After 15 min of incubation, a mean of 97 nmol/ml (two experiments) Hydroquinone was removed and a stoichiometric amount of 1,4-benzoquinone was formed,

as opposed to >91% Hydroquinone being present after 15-min incubation in the absence of hydrogen peroxide. The authors postulated that the selectivity of Hydroquinone may "be due, in part, to the selective ability of macrophages to bioactivate Hydroquinone and/or the selective inability to detoxify 1,4-benzoquinone."

Human promyelocytic leukemia cells (HL-60) were pretreated with noncytotoxic doses of Hydroquinone, 0.1–5 μM, for 4 h to examine the effect of Hydroquinone on the ability of HL-60 to differentiate to marrow stromal macrophage following induction by tissue plasminogen activator (TPA) (Oliveira and Kalf, 1992). A dose-dependent inhibition of differentiation of promyelocytes to marrow stromal macrophages, as measured by the appearance of adherence, nonspecific esterase activity, and the ability to phagocytize sheep erythrocytes was observed. Hydroquinone had no effect on the proliferation of HL-60 cells, and it did not prevent the inhibition of cell proliferation observed after the addition of TPA. Also, Hydroquinone prevented differentiation of HL-60 cells to monocytes/macrophages induced by 1α,25-dihydroxyvitamin D_3 ($1,25\text{-}(OH)_2D_3$), a physiologic inducer. However, Hydroquinone did not have an effect on induction of differentiation to granulocytes by either dimethyl sulfoxide (DMSO) or retinoic acid.

The inhibition of erythropoiesis by Hydroquinone was determined by measuring the incorporation of ^{59}Fe into developing erythrocytes (Bolcsak and Nerland, 1983). Male Swiss–Webster mice were subcutaneously injected with 0.5 ml of 1.3 mmol/kg Hydroquinone in corn oil 12, 24, 48, or 72 h before administration of ^{59}Fe; ten rats per time period were used. The ^{59}Fe uptake assays were performed using a method modified from Lee et al. (1974). Control rats were given pure corn oil.

Administration of Hydroquinone 48 h before ^{59}Fe administration significantly inhibited ^{59}Fe uptake; ^{59}Fe uptake was not inhibited at any other time. Nineteen rats were dosed with 1.2 mmol/kg Hydroquinone 48 h before ^{59}Fe administration; ^{59}Fe uptake was inhibited by 20%.

Twenty rats were dosed with 1.2 mmol/kg Hydroquinone 48 h before ^{59}Fe administration. This group of rats was also given i.p. injections of 400 mg/kg 3-amino-1,2,4-triazole, a benzene oxidation inhibitor, 1 h before test article administration and then every 24 h until ^{59}Fe administration. The inhibition of ^{59}Fe uptake by Hydroquinone was not affected by administration of 3-amino-1,2,4-triazole.

Hydroquinone-induced inhibition of erythropoiesis was investigated using female Swiss albino mice (Snyder et al., 1989). The mice, number per group unspecified, were given i.p. injections of 50, 75, or 100 mg/kg Hydroquinone, 25–100 mg/kg Hydroquinone and 50 mg/kg phenol, 50 mg/kg Hydroquinone and 25–100 mg/kg phenol, or 50 mg/kg Hydroquinone and 1 or 2 mg/kg muconaldehyde 64, 48, and 40 h before administration of 1 μCi of ^{59}Fe. Control mice were given saline. Blood samples were taken 24 h following ^{59}Fe administration. The ^{59}Fe uptake assays were performed using a method modified from Lee et al. (1974, 1981).

Intraperitoneal administration of Hydroquinone, especially 75 and 100 mg/kg, inhibited ^{59}Fe uptake; phenol potentiated this inhibition. Simultaneous adminis-

tration of 50 mg/kg Hydroquinone and 1 mg/kg muconaldehyde significantly decreased uptake of ^{59}Fe (singly, these compounds at the same concentrations did not reduce ^{59}Fe uptake); 50 mg/kg Hydroquinone and 2 mg/kg muconaldehyde completely inhibited ^{59}Fe uptake. The authors concluded that the combined impact of benzene metabolites on various cellular sites may be the cause of failure of bone marrow cell proliferation.

A study performed by Guy et al. (1990) also involved administering Hydroquinone or Hydroquinone and phenol to female Swiss albino mice by i.p. injection 64, 48, or 40 h before i.v. injection of ^{59}Fe (1 μCi/mouse, containing 20–40 ng Fe as Fe citrate). There were six mice per group, and 25–100 mg/kg Hydroquinone, 25–100 mg/kg Hydroquinone and 50 mg/kg phenol, or 50 mg/kg Hydroquinone and 25–100 mg/kg phenol was administered. Control animals were given equal amounts of corn oil or saline. The mice were killed 24 h after ^{59}Fe administration and blood samples were taken. The ^{59}Fe content was determined in a γ scintillation counter.

Hydroquinone, 75–100 mg/kg, decreased iron uptake. Iron uptake was significantly decreased compared with control values at all doses when Hydroquinone and phenol were coadministered, regardless of which compound was held constant and which was varied. The decreased iron uptake caused by the coadministration of Hydroquinone and phenol was greater than the decrease induced by Hydroquinone alone.

P388D1 cells were pretreated with Hydroquinone and Western blot analysis was performed (Renz and Kalf, 1990). At concentrations ≥ 1 μ*M* Hydroquinone for exposure periods of >30 min, interleukin-1-α (IL-1-α) was significantly reduced in cell lysates following LPS stimulation. The reduction of IL-1-α at 1 μ*M* Hydroquinone was not accompanied by a decrease in cell viability, total cellular protein concentration, DNA synthesis, or protein synthesis.

Rat splenic lymphocytes were treated with various concentrations of Hydroquinone and cultured for 3 days in the presence of a T-cell mitogen. Hydroquinone, 10^{-6} *M*, suppressed the lymphoproliferative response (LPR) without being cytotoxic or reducing ATP concentrations within the cells; LPR enhancement was optimal at 10^{-7} *M* Hydroquinone, with near-control LPR values being reached with 10^{-8} *M* Hydroquinone.

NEUROTOXICITY

A study was performed in which a functional–observational battery (FOB) was used to detect functional impairment of the nervous system by Hydroquinone (Eastman Kodak Company, 1988*c*). Sprague–Dawley rats, 10 males and 10 females per group were dosed orally by gavage 5 days per week for 13 weeks with 20, 64, or 200 mg/kg/day Hydroquinone in degassed, distilled water at a concentration of 5%. A group of 10 male and 10 female rats was given a volume of distilled water equal to that of the highest dose group and used as controls.

Clinical observations for each rat were made immediately after dosing; on nondosing days, all animals were observed for mortality. Body weights were determined 3 days before study initiation and body weights and feed consumption were determined on days 0, 1, 4, and 7 and then weekly.

The FOB, which included observational procedures to detect any unusual response concerning body position, degree of activity, coordination of movement, gait, unusual or bizarre behavior, the presence of extraordinary signs, or changes in sensory function, was performed on all animals 3 days before study initiation, at 1, 6, and 24 h after administration of the initial dose. On study days 7, 14, 30, 60, and 91, the FOB was conducted before dosing. Quantitative grip strength was determined when the FOB was performed according to the procedure described by Meyer et al. (1979) and modified by Mattsson et al. (1986). Historical control data were used to demonstrate the sensitivity of the FOB.

At study termination, six males and six females from each group were perfused systematically for neuropathologic examination. The remaining rats underwent necropsy without perfusion. Brain and kidney weights were determined for all animals. Selected tissues from perfused animals were collected for neuropathology analysis.

Treatment-related mortality was not observed during the study. Urine was discolored brown throughout the study for males and females of all dose groups. All animals of the 200 mg/kg dose group and all females of the 64 mg/kg dose group had minimal to minor tremors; seven males of the 64 mg/kg dose group had tremors of minimal severity. Recovery from tremors was rapid. Tremors were observed between 30 min and 1 h after dosing for males and 4–25 min after dosing for females of the 200 mg/kg group. The incidence of tremors was significantly greater for all animals of the 200 mg/kg group during weeks 1–13 and for males of this group during week 14 as compared with controls. For the 64 mg/kg dose group, the incidence of tremors was significantly greater than control values during weeks 6, 7, and 9–13 for males and during weeks 2, 3, 5, 6, 9, and 11–13 for females.

Minimal to mild depression of general activity was statistically greater than that observed for the controls during weeks 1–13 for males and during weeks 4 and 8 for females of the 200 mg/kg dose group; minimal to mild depressed activity was observed for all males and five females. In the 64 mg/kg dose group, minimal depression of general motor activity was observed for one male during week 6, for two males during week 8, and for two females during week 4. Tremors and depressed activity were not observed for the 20 mg/kg dose group. Additional clinical signs were observed, but were not considered dose related.

The mean body weight of the males of the 200 mg/kg dose group was slightly reduced when compared with the mean body weight of controls from day 1 until study termination. This difference was statistically significant at necropsy for perfused males. Feed consumption for males of the 200 mg/kg dose group was statistically less than that of the controls on days 0–4. There were no significant differences in body weight or feed consumption for males in the 20 or 64 mg/kg dose group or any test females when compared with controls.

During FOB examinations, behavioral changes associated with Hydroquinone administration were observed primarily during the 1 and 6 h examinations. For females of the 200 mg/kg dose group, a statistically significant decrease in activity while being removed from the cage was observed at the 1-h FOB. A statistically significant decrease in home cage and locomotor activity was observed for males

of the 200 mg/kg dose group at the 6-h observation. Home cage and locomotor activity was decreased for the males of the 64 mg/kg dose group compared with controls at 6 h, but the decrease was not statistically significant.

Tremors were observed for two males of the 200 mg/kg dose group at the 1-h FOB; although this observation was not significantly different when compared with controls, it was thought to represent a real effect related to Hydroquinone. Tremors were also observed for one male of the 200 mg/kg dose group during the 30-day FOB examination. The incidence of tremors was significantly increased for females of the 200 mg/kg dose group at the 1-h FOB when compared with controls.

The mean forelimb grip strength of females of the 64 mg/kg dose group was significantly less than that of the controls before dosing; no other significant differences in grip strength were observed.

Additional random statistically significant behavioral differences, not related to dose or sex, were also observed; the differences generally did not persist with time.

The mean terminal body weight of perfused males of the 200 mg/kg dose group was significantly less than the controls. There were no significant differences observed for mean brain or kidney weights for dosed animals compared with the controls. No Hydroquinone-related signs were observed at neurologic examination and no lesions were observed at necropsy. There was no evidence that Hydroquinone resulted in lesions of the central or peripheral nervous system. The no-observed-effect concentration of Hydroquinone was 20 mg/kg; central nervous system stimulation was observed with 64 and 200 mg/kg Hydroquinone (Eastman Kodak Company, 1988*c*).

NEPHROTOXICITY

Male and female Fischer-344 (F344) and Sprague–Dawley rats were dosed with Hydroquinone to determine its nephrotoxic effects (Boatman et al., 1992). Three to six rats per sex per group were dosed orally by gavage with 50–400 mg/kg Hydroquinone in degassed water. Indwelling jugular vein cannulas were placed in some rats using a procedure modified from Jongen and Norman (1987); these rats were allowed to recover for 48 h before the test. Another group of rats was pretreated with 10 mg/kg acivicin, a γ-glutamyltransferase (γ-GT) inhibitor, by i.p. injection 1 h before Hydroquinone administration. Two groups of rats were dosed with aqueous solutions of 125 or 250 μmol/kg 2-(glutathion-*S*-yl)hydroquinone via indwelling jugular vein cannulas. Urine and blood samples were obtained at various times for up to 96 h.

Enzymuria and glucosuria were greatest for female F344 rats; however, male F344 rats had a similar pattern. After 8 h, in female F344 rats dosed with 400 mg/kg Hydroquinone, urinary alanine aminopeptidase (AAP), *N*-acetylglucosaminidase (NAG), alkaline phosphatase (ALP), γ-glutamyltransferase (γ-GT), and creatinine values were significantly increased as compared with controls. Urinary NAG values were also significantly increased for female F344 rats given 200 mg/kg Hydroquinone. Urinary glucose and blood urea nitrogen (BUN) values were significantly increased after 24 and 48 h, respectively, for female F344 rats

dosed with 400 mg/kg. For male rats dosed with 400 mg/kg Hydroquinone, urinary AAP, NAG, and γ-GT values were significantly increased compared with controls after 8 h. After 24 h, serum creatinine values were significantly increased for female F344 rats and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values were significantly increased for male F344 rats dosed with 400 mg/kg Hydroquinone.

There were decreases in urinary osmolality with slight diuresis at 8 h for all dosed male and female F344 rats. The decreased osmolality was statistically significant for males and females dosed with 400 mg/kg Hydroquinone. Diuresis was significant for female F344 rats of the 200 or 400 mg/kg dose groups. At microscopic analysis of urine, a significant increase was observed in the epithelial cells (counts/hpf) for female F344 rats dosed with 200 mg/kg Hydroquinone and for male and female F344 rats dosed with 400 mg/kg Hydroquinone as compared with their predose values.

The only significant effect on urinary parameters for Sprague–Dawley rats was that of a decreased BUN value after 48 h for female rats dosed with 400 mg/kg Hydroquinone. No significant changes were observed in measured hematology values. Urinary osmolality and slight diuresis was observed after 8 h for rats of all dose groups.

For cannulated female F344 rats, significant increases were observed in urinary AAP, NAG, ALP, and γ-GT values after 8 h and urinary glucose values after 24 h for those rats dosed with 400 mg/kg and a significant increase was observed in urinary NAG values after 8 h for those rats dosed with 200 mg/kg Hydroquinone as compared with controls. For cannulated male F344 rats dosed with 200 mg/kg Hydroquinone, a significant increase was observed in urinary ALP values after 8 h. Also after 8 h, statistically significant diuresis was observed for female F344 rats dosed with 200 mg/kg and male Sprague–Dawley rats dosed with 400 mg/kg Hydroquinone.

Cannulated male F344 rats dosed with 250 μmol/kg 2-(glutathion-*S*-yl) Hydroquinone had significant increases in urinary AAP, NAG, ALP, and γ-GT values after 8 h and in urinary glucose and BUN values after 24 and 48 h, respectively. These patterns of enzymuria and glucosuria were similar to those observed for male F344 rats dosed with 400 mg/kg. For rats dosed with 250 μmol/kg 2-(glutathion-*S*-yl) Hydroquinone, significant diuresis was observed after 24 h.

For male F344 rats pretreated with acivicin and dosed with 400 mg/kg Hydroquinone, urinary AAP and NAG values were significantly increased after 8 h and urinary γ-GT and BUN values were significantly decreased after 8 and 48 h, respectively, as compared with control values. The increases in AAP and NAG values in the pretreated group were not as great as the increases observed for male F344 rats dosed with 400 mg/kg Hydroquinone without acivicin pretreatment. However, using a ranked data analysis, there was no significant difference in AAP, NAG, or ALP values among the Hydroquinone, acivicin, or Hydroquinone and acivicin groups.

At microscopic examination of the kidneys, alterations included cell regeneration, slight mineralization, slight focal necrosis, and granular casts within the proximal tubules of F344 rats, with the severity and occurrence of these lesions

corresponding to the severity of enzymuria and glucosuria. No significant microscopic observations were made for selected male Sprague–Dawley rats. No increase in the severity of hyaline droplet formation was observed in either strain of rat.

The nephrotoxic effects of Hydroquinone were also evaluated using male and female $B6C3F_1$ mice dosed with 350 mg/kg Hydroquinone in degassed water (Boatman et al., 1992). (The number of mice used was not given.) The dose of 350 mg/kg was chosen because of the high mortality at the dose of 400 mg/kg Hydroquinone. Blood samples from anesthetized mice and urine were collected at various intervals for 48 h.

Urinary creatinine values were significantly increased for male mice after 12 h and urinary BUN values were significantly increased for both male and female mice after 48 h as compared with control values. Because of large standard errors recorded for the measurement of serum values, none of the observed increases were statistically significant. No significant diuresis was observed for the mice.

Male and female F344 and male Sprague–Dawley rats were used to determine whether Hydroquinone induced renal cell proliferation and to study the relationship between the onset of nephrotoxicity and cell proliferation (English et al., 1992*a*). Groups of ten F344 rats, five/sex/group, were dosed orally by gavage with 2.5, 25, or 50 mg/kg Hydroquinone and a group of ten male Sprague–Dawley rats was dosed with 50 mg/kg Hydroquinone in degassed, distilled water at a constant volume of 5 ml/kg. Ten rats of both strains were used as controls and given vehicle only. All rats were dosed 5 days/week, with the F344 rats being dosed for 1, 3, or 6 weeks and the Sprague–Dawley rats being dosed for 6 weeks. Body weights were determined weekly. Urine samples were collected 8 and 24 h after the final dose. At necropsy, the kidneys and a 2 in section of duodenum were preserved.

Osmotic pumps were implanted subcutaneously into the mid-dorsal lumbar area of the rats in order to deliver bromodeoxyuridine (BrDU) continuously for 3 days before killing the animals. Immunohistochemical staining was done using a method modified from Goldsworthy et al. (1991). Sections were examined and scored without knowing the dose given to the animal.

There were no significant differences in body weight and feed consumption between treated and control animals. For male F344 rats, significant increases were observed in urinary ALP values after 1 week of dosing and in urinary AAP and γ-GT values after 6 weeks of dosing with 50 mg/kg Hydroquinone. These changes were considered suggestive of an alteration of the integrity of the proximal tubular membrane and, possibly, necrosis. No other significant changes in urinary parameters were observed for male F344 rats. No significant changes in urinary parameters were observed for female F344 or male Sprague–Dawley rats.

At microscopic examination, renal toxicity was detected in male F344 rats, but not in female F344 or male Sprague–Dawley rats. Tubular degeneration in male F344 rats appeared dose-related. There was no difference observed in the incidence and severity of hyaline droplet formation in the kidneys of treated and control rats.

A significant increase in cell proliferation was observed in tubule segment P2 for female F344 rats dosed for 3 weeks with 2.5 mg/kg ($p < 0.01$) and 50 mg/kg

Hydroquinone ($p < 0.01$; $p < 0.001$); a significant increase was also observed in total cells for female rats of these groups. After 6 weeks of dosing, cell proliferation in tubule segments P1 and P2 and the total cells were significantly increased ($p < 0.01$; $p < 0.001$) for male F344 rats of the 50 mg/kg dose group. For male F344 rats of the 25 mg/kg dose group after 6 weeks, cell proliferation increased 18 and 34% in the P1 and P3 segments as compared with controls; these increases were not considered statistically significant ($p > 0.01$). For female F344 rats after 6 weeks of dosing with 25 mg/kg Hydroquinone, a significant ($p < 0.01$) increase in cell proliferation in tubule segment P1 and in total cells was observed. The authors stated that for female F344 rats, the increases in cell proliferation "were apparently not related to chemical administration, due to the absence of any dose–response relationship." There were no significant differences in cell proliferation between treated and control male Sprague–Dawley rats.

The nuclease P_1–enhanced ^{32}P-postlabeling assay of Reddy et al. (1989), which allows detection at the level of one adduct in 10^9–10^{10} DNA nucleotides, was used to determine whether aromatic DNA adducts derived from Hydroquinone or its metabolites were formed in the kidneys of F344 rats following repeated Hydroquinone administration at doses that were tumorigenic in male rats (English et al., 1992*b*). Three groups, four rats/sex/group, were dosed orally by gavage with 2.5, 25, or 50 mg/kg Hydroquinone in degassed, distilled water at a constant volume of 5 ml/kg, 5 days/week for 5 weeks. A control group of eight rats, four per sex, was given vehicle only. Body weights were determined weekly. Urine was collected at 8 and 23 h after the final dose.

There were no significant differences in the body or kidney weights between treated and control rats. For male rats, significant increases were observed in urinary AAP values at 50 mg/kg, NAG values at 25 and 50 mg/kg, and creatinine values at 2.5 mg/kg Hydroquinone as compared with control values. These differences were suggestive of an alteration in the integrity of the proximal tubular membrane and possibly necrosis. No significant changes in urinary parameters were observed for female rats.

Isolation of DNA from whole kidney homogenates resulted in an alcohol-precipitated DNA pellet that appeared brownish; the brownish discoloration was considered indicative of coprecipitation of impurities. Thin-layer chromatograms of ^{32}P-postlabeled preparations revealed one major spot and several minor spots that were chromatographically dissimilar from the major in vitro adducts of Hydroquinone [and *p*-benzoquinone]. A loss or substantial reduction in "adducts" following repurification of the DNA was considered suggestive of the adducts not arising from Hydroquinone administration.

DNA pellets isolated from kidney nuclei were not discolored. There were no significant differences in the chromatograms of postlabeled DNA from control rats or those of rats dosed with 50 mg/kg Hydroquinone, and no extra spots were observed in the treated chromatograms. There was no exposure-related increase in relative adduct labeling (RAL) values between treated and control samples in areas 6 and 7, where the major in vitro adducts of Hydroquinone [and *p*-benzoquinone] migrate. Also there was no increase in RAL values of other endogenous adducts. Significant reductions were observed in the RAL values of endogenous

adduct spots 2, 3, and 4 and zone 8 in males and spots 1, 4, and 6 and zone 8 in females. Because there were no significant increases in DNA adducts observed for the 50 mg/kg group, DNA adducts from the kidneys of rats of the 2.5 and 25 mg/kg groups were not assayed. The authors concluded that "these data suggest that Hydroquinone produces benign renal tumors in male F344 rats via a non-genotoxic mechanism."

CYTOTOXICITY

The EC_{50} of Hydroquinone was reported to be 0.8 ppm, with a 95% confidence range of 0.5–1.2 ppm (Christian et al., 1976).

The cytotoxicity of Hydroquinone to cultured rat hepatoma cells, HTC line, was examined (Assaf et al., 1986). A dose-dependent response was observed, with a dose of 333 μg/ml, 300 μ*M*, producing 40% cellular mortality after 24 h and 100% cellular mortality after 72 h. A dose of 66 μg/ml, 600 μ*M*, resulted in 100% cellular mortality after 24 h.

The effect of Hydroquinone on human burst-forming unit-erythroid (BFU-E) colony formation by nonadherent bone marrow cells in plasma clots, measured as numbers of benzidine-positive colonies, was examined (Brown et al., 1987). After 7 days of incubation, the cultures were treated with 5×10^{-5} *M* Hydroquinone and maintained for another 7 days. BFU-E growth in the presence of 5×10^{-5} *M* Hydroquinone was 31% of the value obtained for the control cultures. The results of this study indicated cytotoxicity toward stem and stromal cells by Hydroquinone.

The effect of Hydroquinone on stromal cell function was examined by treating mouse bone marrow cells with 10^{-7}–10^{-4} *M* Hydroquinone on culture day 7 (Gaido and Wierda, 1987). After 3 days, the medium was removed and untreated bone marrow cells in agar were plated over the existing stromal cell layer. Stromal cell supported granulocyte/monocyte formation was decreased at concentrations of 10^{-5} and 10^{-4} *M* but increased at a concentration of 10^{-7} *M* Hydroquinone. In a separate set of experiments, 10^{-7}–10^{-5} *M* Hydroquinone, with the same treatment procedure, did not significantly alter total adherent stromal cell number; 10^{-4} *M* Hydroquinone decreased the cell number by 39%.

It was then determined whether the effect of Hydroquinone on stromal cell function was due to prostaglandin synthesis. The cultures were treated using the same procedure as above, with the exception that some cultures were treated with 10^{-6} *M* indomethacin 1 h before Hydroquinone treatment. (This did not completely inhibit prostaglandin synthesis.)

Treatment with Hydroquinone alone produced results similar to those previously reported. Pretreatment with indomethacin significantly reduced the inhibition of stromal cell function by Hydroquinone, but stromal cell function was not enhanced by treatment with 10^{-6} *M* indomethacin followed by 10^{-7} *M* Hydroquinone. Radioimmunoassay (RIA) results were that treatment with increasing doses of Hydroquinone insignificantly increased prostaglandin E_2 (PGE_2) concentrations.

The PGE_2 concentration necessary to significantly inhibit stromal cell-

dependent myelopoiesis was 10^{-7} *M;* increasing doses of Hydroquinone did not increase PGE_2 to inhibitory concentrations. Pretreatment with indomethacin did not significantly decrease PGE_2 concentrations. The authors concluded that the toxicity of Hydroquinone to stromal cells may be due to further activation of Hydroquinone to other toxic moieties within the stroma.

The lymphoma-derived Raji cell line, the erythroleukemia cell line K 562, and the melanotic cell lines IRE 1 and IRE 2 were cultured in the presence of 5×10^{-5}–5×10^{-3} *M* Hydroquinone for 2–48 h (Passi et al., 1987; Picardo et al., 1987). Five experiments were completed in quadruplicate. The survival rates of the cultures treated with 0.1 m*M* Hydroquinone were 62 ± 7–69 ± 6% after 24 h; the cell line K 562 was least affected and cell line IRE 1 was most affected. Treatment with 0.1 m*M* Hydroquinone for 24 h resulted in survival rates of 20 ± 4–24 ± 4%, with cell line IRE 2 being least affected and Raji cell line being most affected. Preincubation with Hydroquinone in the medium for 24 and 48 h decreased the cell survival rate; addition of oxygen radical scavengers to the medium, singly or in combination, increased cell survival rates.

Day 5 macrophage cultures, day 3 LTF cultures, and day 14 primary fibroblast cultures were each exposed to various doses of Hydroquinone for 48 h; cell viability was then determined (Thomas et al., 1989). Macrophage viability was significantly reduced at doses of $\geq 10^{-5}$ *M* Hydroquinone; some cell death was observed with 10^{-6} *M* Hydroquinone. LTF cell viability was only affected at a dose of 10^{-4} *M* Hydroquinone; 10^{-6} *M* Hydroquinone did not affect innate colony-stimulating activity of primary fibroblast cell cultures.

In order to determine whether a decreased support of myelopoiesis by bone marrow cells after in vitro treatment with Hydroquinone was due to toxicity of bone marrow macrophages or fibroblastoid stromal cells, macrophages were exposed to 10^{-8}–10^{-4} *M* Hydroquinone and LTF cultures were exposed to 10^{-7}–10^{-4} *M* Hydroquinone for 48 h. LTF cells were reconstituted with treated macrophages. The reconstituted cultures were cocultured after 24 h and the number of granulocyte/macrophage colonies (G/M-CFU-C) that formed after 7 days was determined.

Hydroquinone reduced macrophage activity at a concentration of 10^{-7} *M;* significant reductions were observed at doses of $\geq 10^{-6}$ *M*. Treated LTF cells that were reconstituted with untreated macrophages and then cocultured were not significantly affected by Hydroquinone. When LTF cells were treated with 10^{-4} *M* Hydroquinone, a decrease in constitutive production of colony-stimulating activity was observed.

Cultures of primary fibroblastoid cells isolated from fresh bone marrow adherent cells containing >95% fibroblastoid cells and macrophages were reconstituted with Hydroquinone-treated macrophages and cocultured. Reconstitution with macrophages that were treated with 10^{-6} *M* Hydroquinone inhibited production of colony-stimulating activity and a significant decrease in the ability to support G/M-CFU-C formation was observed.

To determine the effect of Hydroquinone on macrophage interleukin-1 (IL-1) production, IL-1 activity was measured in conditioned medium that was removed from Hydroquinone-treated LPS-stimulated macrophage cultures. A dose of 10^{-6}

M Hydroquinone significantly reduced IL-1 activity. The authors concluded that Hydroquinone specifically interferes with bone marrow stromal macrophage activity, possibly due to a reduction in IL-1 activity (Thomas et al., 1989).

Hydroquinone, 1 μ*M*–1 m*M*, was added to keratinocytes in culture (Picardo et al., 1990*a*). Cytotoxic activity was observed at concentrations >10 μ*M*, with the effect being correlated to the rate of decomposition. The presence of oxygen radical scavenger enzymes significantly reduced the toxicity. A significant release of arachidonic acid from cell membranes was observed after 1–2 h incubation with >100 μ*M* Hydroquinone. Hydroquinone "may produce a toxic effect on human keratinocytes, partially due to the production of oxygen radicals and to the generation of oxidation products. These phenomena may induce the release of lipooxygenase substrates and lead to inflammatory reactions."

Bone marrow cells from the femurs of male C57Bl/6J and DBA/2J mice were used to establish primary adherent stromal cell cultures; the cultures were treated with various concentrations of Hydroquinone for 24 h (Twerdok and Trush, 1990). Stromal cells derived from DBA/2 mice were significantly more sensitive to Hydroquinone than the cells from C57Bl/6 mice; the lethal concentration$_{50}$ (LC_{50}) for DBA/2 cells was 49.2 ± 2 μ*M* and for C57Bl/6 cells was 95.3 ± 13.5 μ*M*. Induction of quinone reductase by *t*-butylhydroquinone before treatment with Hydroquinone protected against Hydroquinone toxicity.

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

Groups of 4–20 rats, equal number of males and females per group, were dosed orally with fresh preparations of 180–2,100 mg/kg Hydroquinone or 1-week-old preparations of 420–940 mg/kg Hydroquinone as 5% aqueous solutions (Christian et al., 1976). The animals were observed for 3 weeks for adverse effects and mortality. The oral LD_{50} was determined to be 743 and 627 mg/kg for male and female rats, respectively. All adverse effects were almost immediate (usually within 2–10 min) upon dosing. Clinical observations included hyperactivity and hypersensitivity to auditory and tactile stimuli, tremors, moderate clonic convulsions, severe tonic spasms, protuberant and dark eyes, and a discoloration of the nose, lips, and paws. Generally, mortality occurred within 2 h after dosing. After 24 h, some animals were still hypersensitive to tactile stimuli and had a transient decrease in body weight. All surviving animals were normal within 3 days after dosing. Results were similar for both fresh and aged solutions.

In a metabolic study (described earlier), oral administration of 350 mg/kg Hydroquinone by gavage resulted in reduced activity in male rats (Eastman Kodak Company, 1988*b*). Female rats had mild tremors with chewing and minimal reduced activity during a 15–45-min period after test article administration. At 3.5–4.0 h after dosing, the animals appeared normal. No adverse effects were observed following dosing with 25 mg/kg Hydroquinone.

The approximate lethal oral dose of Hydroquinone was 0.2 g/kg for rabbits and 0.08 g/kg for cats (Deichmann, 1983).

Short-Term Toxicity

Oral

Male and female rats, six/sex/group, were dosed with 2,500–10,000 ppm Hydroquinone in drinking water for 8 weeks (Christian et al., 1976). A control group was given drinking water only. Clinical observations were made daily. Body weights and feed consumption were measured weekly; water was changed and consumption was measured three times weekly. Changes in microsomal hepatic enzymes were evaluated by measuring the length of the period of sleep induced by an i.p. injection of 125 mg/kg sodium hexobarbital; sleep time was considered the timed interval between loss and regaining of the righting reflex. Blood samples were taken during the study. At the termination of dosing, the animals underwent necropsy.

There was no mortality during the study, and no adverse clinical observations were noted. Growth was decreased for all rats of the 10,000 ppm and for female rats of the 5,000 ppm dose groups. There was no significant difference in feed consumption between treated and control rats; water consumption decreased proportionate to increased doses of Hydroquinone. There were no significant differences in hematologic parameters between treated and control animals. For male rats, the period of sleep induced by sodium hexobarbital decreased with increasing doses of Hydroquinone; this effect was not seen for female rats. Absolute liver and kidney weights of animals dosed with Hydroquinone increased with increasing concentrations of Hydroquinone, with the increased liver weights being significant for male rats of the 5,000 ppm dose group and male and female rats of the 10,000 ppm dose group. Organ-to-body weight ratios were significantly increased for the livers and kidneys of all rats of the 5,000 and 10,000 ppm dose groups and for the kidneys of female rats of the 2,500 ppm dose group. No significant microscopic observations were made.

F344/N rats and B6C3F$_1$ mice, five/sex/group, were dosed with Hydroquinone in corn oil by gavage 5 days/week; 12 doses were given over 14 days (NTP, 1989; Kari et al., 1992). The rats were dosed with 63, 125, 250, 500, or 1,000 mg/kg and the mice with 31, 63, 125, 250, or 500 mg/kg Hydroquinone. A group of rats and a group of mice were dosed with vehicle only and served as controls. All animals were observed daily and body weights were measured on days 0, 7, and 14. The animals were fasted overnight and killed for necropsy.

All male and female rats of the 1,000 mg/kg group and one male and four females of the 500 mg/kg group died on study. One male of the 1,000 mg/kg group died accidentally. Four male and five female mice of the 500 mg/kg group and two male mice of the 250 mg/kg group died on study. A few other mice died because of gavage accidents.

Tremors, which lasted $\leq$30 min, were observed for rats of the 500 and 1,000 mg/kg groups. For male rats of the 1,000 mg/kg group, the tremors were followed by convulsions and death. For the male and female mice of the 500 mg/kg group

and male mice of the 250 mg/kg group, tremors, followed by either recovery or convulsions and death, were observed. Tremors followed by recovery were observed for female mice of the 250 mg/kg group.

Dermal

F344/N rats and B6C3F$_1$ mice, five/sex/group, received 12 dermal applications of Hydroquinone in 95% ethanol over 14 days (NTP, 1989). The test article was applied to a clipped area of the scapular region. The rats were dosed with 240, 480, 960, 1,920, or 3,840 mg/kg and the mice with 300, 600, 1,200, 2,400, or 4,800 mg/kg Hydroquinone. The high dose was administered to rats and mice in two portions, with a 15–30-min interval for the test material to dry. A group of rats and a group of mice were dosed with vehicle only and used as controls. All animals were observed daily and weighed on days 0, 7, and 14. The animals were fasted overnight and killed.

No rats or mice died on study. There was no significant difference in weight gain between treated and control animals. In both the rat and mouse high-dose groups, crystals were observed on the skin and hair.

Intraperitoneal

Six male albino Swiss mice were given i.p. injections of 10 mg/kg Hydroquinone in saline 6 days/week for 6 weeks (Rao et al., 1988). A control group of six male mice was given i.p. injections of saline for the same time period. Following the last dose, the animals were weighed and killed, with blood samples being taken. All animals underwent necropsy and certain tissues were examined microscopically. Suspensions of bone marrow cells were made.

No dose-related deaths occurred on study. There were no significant differences in hemoglobin, total leukocytes, lymphocyte, polymorph, or lymphocyte/polymorph ratio values when compared with controls. Hydroquinone produced an insignificant depression in bone marrow cellularity. Microscopically, the livers of animals dosed with Hydroquinone had a loss of cytoplasmic detail of hepatocytes and focal fatty change. Hydroquinone did not produce a change in body weight or relative organ weight when compared with the controls. The adrenal glands of treated mice had moderate congestion at the corticomedullary junction. The study size did not warrant the drawing of conclusive results.

Subchronic Toxicity

Oral

Male and female rats, 20/sex/group, were dosed with 1,000–4,000 ppm Hydroquinone in drinking water for 15 weeks (Christian et al., 1976). A control group was given drinking water only. Clinical observations were made daily. Body weights and feed consumption were measured weekly; water was changed and consumption was measured three times weekly. Changes in microsomal hepatic enzymes were evaluated by measuring the length of the period of sleep induced by an i.p. injection of 125 mg/kg sodium hexobarbital; sleep time was considered the

timed interval between the loss and regaining of the righting reflex. Blood samples were taken during the study. At the termination of dosing, the animals underwent necropsy.

There was no mortality during the study, and no adverse clinical observations were noted. Growth was significantly decreased for male rats of the 4,000 ppm dose group. Males and females of the 1,000 ppm group generally grew faster and consumed more feed than other males and females. Water consumption decreased proportionate to increased doses of Hydroquinone. The only differences noted in hematologic parameters between treated and control animals were slightly reduced hematocrits and hemoglobin contents after 5 and 10 weeks for rats dosed with 2,000 and 4,000 ppm; these values were normal at study termination. The period of sleep induced by sodium hexobarbital was not affected by Hydroquinone administration. Absolute liver and kidney weights of animals dosed with Hydroquinone increased with increasing concentrations of Hydroquinone. Liver-to-body weight ratios were significantly increased for male and female rats of all dose groups; kidney-to-body weight ratios were significantly increased for male rats of all dose groups and for female rats of the 2,000 and 4,000 ppm dose groups. No significant microscopic observations were made.

F344/N rats and B6C3F_1 mice, 10/sex/group, were dosed with Hydroquinone dissolved in corn oil by gavage 5 days/week for 13 weeks (NTP, 1989; Kari et al., 1992). The doses for both rats and mice were 25, 50, 100, 200, and 400 mg/kg Hydroquinone. A group of rats and a group of mice were dosed with vehicle only and served as controls. Animals were observed twice daily and body weights and feed consumption were measured weekly. All animals were killed for necropsy at the termination of dosing. Tissues of rats and mice of the control, 200, and 400 mg/kg groups and selected tissues from rats of the 100 mg/kg group were examined microscopically.

All the rats of the high-dose group and three female rats of the 200 mg/kg group died on study, with most of the deaths occurring before week 7. In the 200 mg/kg dose group, male and female rats appeared lethargic after 10 and 11 weeks of dosing, respectively. Tremors, sometimes followed by convulsions, were observed in female rats at this dose. For the rats that died, tremors and convulsions were often observed before death, and there was a clear orange fluid or orange staining around the mouth. No clinical signs were observed in the other dose groups.

Relative liver-to-body weight ratios were significantly decreased for male rats in all dose groups and were significantly increased for female rats of the 100, 200, and 400 mg/kg groups when compared with control values.

Four male and five female rats of the high-dose group had red-to-brown perioral staining, one male and two females had reddened gastric mucosa, and one male had meningeal hemorrhage. In the 200 mg/kg group, two females had perioral staining, two males had intra-abdominal bleeding, one female had blood in the gastric contents, seven males and six females had toxic nephropathy, and four males and one female had inflammation and mild to moderate hyperplasia of the squamous portion of the stomach. Toxic nephropathy was observed in one female rat of the 100 mg/kg group. The renal lesions consisted of tubular cell degeneration

and degeneration in the renal cortex and were of moderate to marked severity in the male and of minimal to mild severity in the female rats.

Eight male and eight female mice of the high-dose group and two male mice of the 200 mg/kg group died on study; the death of one male mouse of the 200 mg/kg group mice was accidental. Lethargy was observed in all dosed males and in females of the three highest dose groups. Post-dose tremors, often followed by convulsions, were observed in males and females of the high-dose group. Post-dose tremors were also observed in males of the 200 mg/kg dose group. For male mice of all dose groups and female mice of the two highest-dose groups, relative liver-to-body weight ratios were significantly increased compared with control values. Ulceration, inflammation, or epithelial hyperplasia of the squamous portion of the stomach was observed in three male and two female mice of the high-dose group and in one female mouse of the 200 mg/kg group.

Dermal Irritation

In a metabolic study (described earlier), dermal application of 25 or 150 mg/kg Hydroquinone to male and female Fischer 344 rats for 24 h produced slight to severe erythema due to crystallization and wiping (Eastman Kodak Company, 1988*b*).

Sensitization

The sensitization potential of Hydroquinone was examined in both a Magnusson–Kligman guinea pig maximization test and a local lymph node assay (LLNA), which is a screening assay (Basketter and Scholes, 1992). Albino Dunkin–Hartley guinea pigs were used in the maximization test. Following a preliminary irritation test to determine test concentrations, the animals received a series of six intradermal injections of 2.0% Hydroquinone in 0.9% saline in the shoulder region to induce sensitization. After 6–8 days, a 48-h occlusive patch of 1.0% Hydroquinone in acetone–polyethylene glycol 400 was placed over the injection site. The animals were challenged on a previously untreated area of the flank using a 24-h occlusive patch of 0.5% Hydroquinone, which was the maximum nonirritating concentration. The challenge site was scored for erythema and edema 24 and 48 h after patch removal. Hydroquinone was classified as an extreme sensitizer, with 100% of the animals having a positive response after 14 and/or 48 h.

The LLNA was performed as described by Basketter et al. (1991). Male and female CBA/Ca mice, four per group, were used; single experiments were limited to one sex. The mice were dosed daily with a topical application of 25 μl of 0.5, 1.0, and 2.5% Hydroquinone in acetone–olive oil (4:1, v/v) on the dorsal surface of each pinna for 3 consecutive days. Control mice were dosed with vehicle only. Four days after the first application, all mice were given an i.v. injection of 250 μl phosphate-buffered saline (PBS) containing [^{3}H]methyl thymidine (^{3}HTdR; 20 μCi). The mice were killed 5 h after i.v. dosing, the draining auricular lymph nodes were excised and pooled for each group, and a single-cell suspension of lymph node cells (LNC) was prepared. ^{3}HTdR incorporation was measured using β-scintillation counting. A chemical was considered a sensitizer if at least one

concentration resulted in at least a threefold increase in ^{3}HTdR incorporation as compared with control values. Hydroquinone was positive for sensitization.

Reproductive/Teratogenic Effects

The developmental toxicity potential of Hydroquinone was evaluated using pregnant Crl: COBS CD (SD)BR rats (Eastman Kodak Company, 1985*d;* Krasavage et al., 1992). Three groups of rats, 30 per group, were dosed orally by gavage with 30, 100, or 300 mg/kg Hydroquinone, 5% in distilled water, on days 6–15 of gestation. A group of 30 rats received a volume of distilled water equivalent to the largest volume of Hydroquinone solution given to a test animal on days 6–15 of gestation and served as a control group. Body weights and feed consumption were determined on days 0, 6, 9, 12, 16, and 19 of gestation. All animals were observed three times a day on dosing days or twice daily on nondosing days, respectively. On weekends during the nondosing periods, the animals were observed for morbidity and mortality. On day 20 of gestation, fasted animals were killed and examined. The liver and kidneys of each animal were weighed and examined microscopically.

No significant differences were observed in maternal mean body weight, corrected mean body weight, absolute organ weight, or feed consumption. Any statistically significant increases in relative organ weight were not considered toxicologically important. The only clinical observation for all animals dosed with Hydroquinone was brown discolored urine. No Hydroquinone-related lesions were observed during gross examination for any dose group, and no abnormalities were observed upon microscopic examination of the liver and kidneys of animals of the high-dose group. (The liver and kidneys of animals of the other dose groups were not examined microscopically.)

Combined male and female fetal mean body weight was significantly reduced for the 300 mg/kg dose group compared with controls. When measured separately, female fetal mean body weight was significantly less and male fetal mean body weight was slightly but not significantly less than control values. There was no significant difference in malformations or skeletal alterations between fetuses in the dose and control groups.

The no observable effect level (NOEL) for both maternal and developmental toxicity was 100 mg/kg Hydroquinone. The no observable adverse effect level was 300 mg/kg. Hydroquinone, $\leq$300 mg/kg, did not produce embryotoxic, fetotoxic, or teratogenic effects in Crl: COBS CD (SD)BR rats.

A two-generation study was conducted using Charles River CD (Sprague–Dawley)-derived rats to determine the reproductive effects of long-term oral administration of Hydroquinone (Bio/Dynamics, Inc., 1989*a;* Blacker et al., 1993). The F_0 generation consisted of 30 males and 30 females per group which were dosed with 15, 50, or 150 mg/kg Hydroquinone in 5 ml/kg degassed distilled water at concentrations of 3, 10, or 30 mg/ml, respectively, by gastric intubation 7 days/week for 10 weeks before mating. A control group of 30 males and 30 females was dosed with 5 ml/kg of vehicle.

One male was housed nightly with one female from the same dose group until

evidence of mating was observed or until 7 days had elapsed without evidence of mating. Unmated females were then reassigned to sexually active males of the same dose group for another 7-day period. F_0 males were dosed daily during mating and the postmating period until they were killed. Mated F_0 females were dosed daily during mating and during the gestation and lactation periods. Unmated females were dosed during the postmating period until they were killed.

Two pups/sex/litter were randomly chosen at weaning (day 24) to be used as the F_1 parent generation. Excess pups were culled to 30 males and 30 females per group in a way that allowed each litter to be represented by at least one pup/sex. Rats chosen for the F_1 parent generation were administered the same dose as their parents starting on day 25 of age; however, the premating dose period of 7 days/week for 11 weeks did not formally begin until the last litter was weaned. The F_1 animals were mated and dosed using the same procedure as that used for mating F_0 animals. (Brother–sister mating was avoided.)

Animals were observed twice daily for mortality, toxicologic and pharmacologic effects, and parturition. All animals were examined in detail weekly for signs of local or systemic toxicity or pharmacologic effects. From week 8 until termination of study, F_0 and F_1 parents were observed daily for tremors following dosing.

Body weights of the F_0 and F_1 parents were measured at the time of assignment to the test group, the day of dose initiation, day 4 of dosing, and weekly during the premating period. Males were weighed weekly until killed. Female body weights were measured on days 0, 7, 14, and 20 of gestation and on days 0, 4, 14, and 21 of lactation. Feed consumption was measured weekly during the premating period for all F_0 and F_1 parents. For males, feed consumption was also measured weekly during the postmating period. For females, feed consumption was measured for the intervals gestation days 0–7, 7–14, and 14–20.

Litters were examined twice daily. Pups were counted, the number of pups/sex was determined, gross physical examinations were made, and body weights of live pups were measured on days 0, 4, 7, 14, 21, and 24 of lactation for the F_1 litters and on days 0, 4, 7, 14, and 21 of lactation for the F_2 litters. On day 4 of lactation, each litter was culled to eight pups, four males and four females, when possible.

Parent females were killed as a group after the weaning of the last litter. F_0 and F_1 adult males were killed 4 and 3 weeks, respectively, after mating period completion. F_1 pups not selected for the pool of animals from which the F_1 parent generation was to be chosen were killed on day 24 of lactation. F_1 pups chosen for the pool but not chosen for the F_1 parent generation were killed at weaning of the last litter. F_2 pups were killed on day 21 of lactation. Gross examination was performed on all animals. Various tissues from all F_0 and F_1 parent animals were preserved. The tissues of the control and 150 mg/kg groups were examined microscopically. For the 15 and 50 mg/kg dose groups, tissues with gross lesions and reproductive tissues from sexually inactive animals were examined microscopically.

There was no significant difference in mortality rates between F_0 and F_1 parents and the controls. In the 150 mg/kg group, one female of the F_0 generation and two females of the F_2 generation had tremors before death.

Mean body weight, mean body weight gains, and mean feed consumption for all dose groups of both the F_0 and F_1 generations, males and females, were comparable to control values during the premating period.

No toxicologically significant differences were observed in the weekly mean body weights or mean feed consumption for all F_0 and F_1 males during the mating and postmating periods. Maternal mean body weights and mean body weight gains were comparable between treated and control animals of the F_0 and F_1 generations during gestation and lactation and maternal mean feed consumption during gestation was also comparable for treated and control groups.

Tremors were observed in some animals after dosing. The occurrence of tremors in one F_0 male of the 50 mg/kg dose group and in F_0 and F_1 males and females of the 150 mg/kg group dose was considered indicative of an adverse effect.

No adverse effects related to Hydroquinone administration were observed at necropsy or during microscopic evaluation of either the F_0 or F_1 animals. The NOEL for parental effects was 15 mg/kg/day.

There were no significant differences in mating indices, pregnancy rates, or male fertility indices between the treated and control males and females. The mean gestation length for the treated F_0 and F_1 females was comparable to the controls. The number of live, dead, and total pups at birth, pup viability indices, and mean litter size at day 4 (pre-cull) and at post-cull intervals for both generations were comparable between treated and control groups. Litter survival indices were also comparable between treated and control groups of both generations.

No significant difference was observed in pup mean body weights or sex distribution between treated and control groups of either generation. There was no significant difference for either generation in the number of external or internal malformations for treated and control pups, and adverse effects of dose administration were not apparent. The NOEL for reproductive effects was 150 mg/kg/day.

A range-finding study was conducted to assess the toxicity of Hydroquinone using pregnant New Zealand White (NZW) rabbits (Bio/Dynamics, Inc., 1988; Murphy et al., 1992). Five rabbits per group were given 50, 100, 200, or 400 mg/kg Hydroquinone in 10 ml/kg distilled, degassed water at a concentration of 5, 10, 20, or 40 mg/ml, respectively, by gastric intubation on days 6–18 of gestation. Initially, a group was given 500 mg/kg Hydroquinone at a concentration of 50 mg/ml, but this group was eliminated and replaced by five rabbits receiving 300 mg/kg Hydroquinone, at 30 mg/ml, on days 6–18 of gestation following the death of the first two animals dosed with 500 mg/kg Hydroquinone. A control group of five rabbits was dosed with 10 mg/ml of vehicle on days 6–18 of gestation.

All animals were observed for pharmacologic and toxicologic effects and mortality twice daily and examined in detail on days 0, 6, 9, 12, 15, 18, 24, and 30 of gestation. Body weights were determined on days 0, 6, 12, 18, 24, and 30 and feed consumption on days 3, 6–10, 12, 15, 18, 24, and 29 of gestation. All animals were killed on day 30 of gestation.

Mortality was not observed in the control, 50, 100, or 200 mg/kg dose groups. There was 100% mortality in the 400 mg/kg dose group within 2–4 days after dose

initiation and in the 300 mg/kg dose group by week 13. As was mentioned earlier, the two animals dosed with 500 mg/kg Hydroquinone died after the first dose.

Hydroquinone administration did not have an adverse effect on pregnancy rate for the animals of the 50, 100, or 200 mg/kg dose groups. Pregnancy rates of the 300, 400, and 500 mg/kg dose groups were decreased compared with the controls. Some animals may have died before implantation occurred. The absence of implants in some females that died later in the gestation period may be suggestive of an adverse effect on the uterine implantation process or an embryolethal effect.

No significant effects on weight gain or mean feed consumption were observed for animals of the 50 mg/kg group, but significant effects were observed for animals of the 100, 200, and 300 mg/kg dose groups. Using corrected body weights, a mean weight loss was observed during the gestation days 6–30 interval for animals of the control, 50, 100, and 200 mg/kg groups.

Tremors were observed on day 1 for some animals dosed with 300, 400, and/or 500 mg/kg Hydroquinone. Excessive lacrimation was observed in four animals of the 300 mg/kg dose group at various times during dosing and may have been suggestive of a treatment-related effect. Dark brown urine was observed for some animals in all dose groups. Other observations were not considered dose related.

Red discoloration of the gastric mucosa and changes in the gastric contents were observed for some animals of the 300, 400, or 500 mg/kg dose groups. Yellowish/brown material stained the anogenital skin/hair of animals of these groups also. Other gross observations made for treated animals at necropsy were comparable to those made for controls.

No adverse effects, such as abortion or premature delivery, were observed for animals dosed with $\leq$200 mg/kg Hydroquinone. The mean number of corpora lutea and uterine implantation sites, mean preimplantation loss index, mean number of live fetuses per pregnant female, and the incidence of litters containing at least one resorption site were similar among the control, 50, 100, and 200 mg/kg groups. An increase in the mean number of resorption sites and the mean ratio of resorptions to implants for the 200 mg/kg group as compared with the controls may have been indicative of a treatment-related response.

Fetal mean weights were similar for the control, 50, and 100 mg/kg groups, but decreased for the 200 mg/kg dose group, suggestive of a treatment-related effect. No adverse effects due to Hydroquinone were observed during external evaluation of the fetuses of the 50, 100, and 200 mg/kg dose groups.

A study was conducted to evaluate the embryotoxic, fetotoxic, and/or teratogenic potential of Hydroquinone using NZW rabbits (Bio/Dynamics, Inc., 1989*b;* Murphy et al., 1992). Three groups of 18 rabbits were given 25, 75, or 150 mg/kg Hydroquinone in 8 ml/kg degassed, distilled water at a concentration of 3.125, 9.375, or 18.75 mg/ml Hydroquinone, respectively, by gastric intubation on days 6–18 of gestation. (These doses were selected based on the results of the range-finding study that was described previously.) Eighteen rabbits were dosed with 8 mg/ml of vehicle on days 6–18 of gestation and served as a control group.

All animals were observed for pharmacologic and toxicologic effects and mortality twice daily, and examined in detail on days 0, 6–19, 24, and 30 of gestation. Body weights were determined on days 0, 6, 9, 12, 16, 18, 24, and 30, and feed

consumption was recorded daily during gestation. All animals were killed on day 30 of gestation.

There was no mortality in the control or treated groups. There was no significant difference in body weights observed between the 25 and 75 mg/kg dose groups and the controls. For the 150 mg/kg group, the mean body weight was significantly lower than the control group mean body weight on days 16 and 18 of gestation and a mean weight loss was significant during the days 6–9 interval. The total mean weight loss over the entire dosing period was significantly greater than the total mean weight loss observed for the control group. Corrected gestation day 30 body weights were comparable among the treated and control groups.

Mean feed consumption of the 25 mg/kg dose group was comparable to control values at all times whereas it was significantly decreased for the 75 mg/kg dose group on days 11 and 12 and for the 150 mg/kg dose group on days 6–14 and 17 compared with controls.

No adverse effect of treatment was evident upon physical observation. A dark-staining urine was noted for many of the treated animals.

The pregnancy rates, premature delivery indices, mean number of corpora lutea, and mean number of uterine implantations per pregnant female were comparable between the control and treated groups. The mean preimplantation loss indices and mean number of viable fetuses were comparable between the control, low-, and high-dose groups, but the preimplantation loss index was considerably decreased and the mean number of viable fetuses was increased for the mid-dose group as compared with controls. These differences were not considered indicative of an adverse effect of dosing. The mean number of resorptions per pregnant female, mean resorption/implant ratio, and the mean number of females with resorptions among their uterine implants were comparable among all groups.

At necropsy, an accentuated lobular pattern of the liver was noted for some control and treated animals, with the incidence being greater in the low- and mid-dose groups as compared to controls. The toxicologic significance of this observation, especially without a dose relation, was equivocal on the basis of gross examination only. Other gross changes were not considered dose related. Absolute and relative kidney and liver weights were comparable among all animals.

No significant differences in fetal mean weights or sex distribution were observed between the control and treated groups.

The incidence of external, visceral, or skeletal malformations or ossification variations, both per fetus and per litter, was not significantly different between the control and treated groups. Dose-related external or visceral variations were not observed.

MUTAGENICITY

Results of mutagenicity studies that have been published since 1983 are essentially the same as those reported in the existing safety assessment of Hydroquinone (Elder, 1986), and are summarized in Table 2.

The results of Ames tests with and without metabolic activation using *Salmo-*

TABLE 2. *Hydroquinone (HQ) mutagenicity studies*

Test	Organism and strain	Methods	Results and comments	Reference
Ames test	*S. typhimurium* TA1535, TA1537, TA98, TA100	At least five concentrations were tested with or without S9. The experiment was run in triplicate. Positive and negative controls were used	Negative	Haworth et al., 1983
Ames test	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535	HQ at concentrations of 50–1,000 and 1.25–40 μg/plate was tested with and without S9, respectively	Negative	Glatt et al., 1989
Ames test	*S. typhimurium* TA97, TA98, TA100	HQ and HQ plus nitrite at concentrations of 12.5–100 μg/plate and HQ plus chlorine at concentrations of 1–25 μg/plate were tested with and without S9. The vehicle, DMSO, was used as the negative control. The experiment was run in triplicate. The ability of HQ to produce H_2O_2 and O_2^- was also examined	HQ was a direct-acting mutagen toward all three strains, with the chlorinated and nitrosated derivatives being more mutagenic than HQ alone. Mutagenic activity was greatly suppressed in the presence of S9. 100 μg/plate HQ was toxic to primarily all cultures.HQ was able to produce H_2O_2 but not O_2^-. The amount of H_2O_2 produced was decreased in the presence of catalase	Lin and Lee, 1992
Salmonella mutagenicity test	*S. typhimurium* TA97, TA98, TA100	Mutagenic potential was assayed at a dose range of 0–250 μg/plate with and without S9 according to the method of Maron and Ames (1983). Results were expressed as an average of two plates/dose	Negative	Sakai et al., 1985
Fluctuation test	*S. typhimurium* TA100	Mutagenic potential, with and without metabolic activation, was assayed using the method modified by Gatehouse	Positive with metabolic activation. Negative without metabolic activation	Koike et al., 1988
6-TG resistance induction	V79 Chinese hamster cells	At least five concentrations of HQ were tested; the optimal/maximal concentration was 3.5 μ*M*	Positive	Glatt et al., 1989
Promutagenic chemical detection	*S. griseus* H69, FS2	*S. griseus* strains H69 (detects point mutations) and FS2 (detects frame shift mutations) were used to assess the activation of promutagenic chemicals by monitoring the reversion of the bacterial test strains to a kanamycin-resistant phenotype. HQ was tested at a concentration of 200 μg/ml	Negative with both strains	Buchholz et al., 1992

(continued)

TABLE 2. *Continued*

Test	Organism and strain	Methods	Results and comments	Reference
Sister chromatid exchange	CHO cells	Dose ranges of 50–800 and 0.5–5 μg/ml were tested with and without S9, respectively. Positive and negative controls were used	Positive	Galloway et al., 1987
Sister chromatid exchange	Chinese hamster Don cells		Positive	Shimada et al., 1988
Sister chromatid exchange	V79 Chinese hamster cells	The optimal/maximal concentration was 20 μ*M*	Positive	Glatt et al., 1989
Sister chromatid exchange	Human lymphocytes	Lymphocytes from whole blood of 7 individuals were cultured with 40 μ*M* HQ, 40 μ*M* HQ, and 1–100 μ*M* acetaldehyde, or 50 μ*M* dimethyl maleate and 40 μ*M* HQ	HQ alone significantly increased the number of SCEs in cultures from 3 of 7 of the individuals. A synergistic effect was observed when acetaldehyde (at concentrations which alone did not increase SCEs) was administered with HQ. Pretreatment with dimethyl maleate more than doubled the number of SCEs	Knadle, 1985
Chromosomal aberration	*A. nidulans*	A concentration range of 250–750 μg/ml was tested. Minimal and complete media were used and ≥100 colonies were tested at each concentration	Mitotic segregation was greatly increased. Haploid segregation was only slightly increased	Kappas, 1989
Chromosomal aberration	*A. nidulans*	A concentration range of 132–396 μg/ml HQ in distilled water was tested. A positive control was run. Minimal and complete media were used	HQ significantly increased whole chromosome segregants	Crebelli et al., 1991
Chromosomal aberration	*A. nidulans*	HQ, 1–3 m*M*, was tested without metabolic activation. The effect of 2 m*M* L-cysteine and 2 m*M* HQ was also examined	HQ produced an 8- to 10-fold increase in mitotic segregation. L-cysteine lowered the frequency of abnormal colonies to control values	Crebelli et al., 1987
Chromosomal aberration	*A. nidulans*	A concentration range of 250–750 μg/ml was tested without metabolic activation	HQ produced a 2- to 5-fold increase in the number of diploid mitotic crossover segregants. Haploid and nondisjunctional diploids were not increased	Kappas, 1990
Chromosomal aberration	CHO cells	Dose ranges of 150–600 and 5–20 μg/ml were tested with and without S9, respectively. Positive and negative controls were used	Positive with S9. Negative without S9	Galloway et al., 1987
Chromosomal aberration	Chinese hamster cell lines: immortal		*Don.Wg3H:* HQ induced metaphase, anaphase, and telophase defects and the absence of mitotic spindle	Parry et al., 1990

	Don.Wg3H; primary LUC1		*LUC1:* HQ induced metaphase and anaphase defects and the absence of mitotic spindle	
Chromosomal aberration	*A. nidulans*	A concentration range of 132–396 μg/ml HQ in distilled water was tested. A positive control was run. Minimal and complete media were used	HQ increased the frequency of mitotic crossovers	Crebelli et al., 1991
Aneuploidy induction	Mouse testicular cells	HQ in double-distilled water at concentrations of 80–120 mg/kg was given by i.p. injection and slides were prepared after 6, 14, or 22 h. From each animal, 100 metaphase II cells were examined for hypo- and hyperploidy. Negative controls were used	The frequency of hyperploid meiotic metaphase II (MMII) cells was significant at a dose of 100 mg/kg HQ after 6 h. When all dose groups were compared with the controls by the trend test, HQ caused significant induction of aneuploidy. The frequency of hypoploid MMII cells was not significantly increased. HQ induced nondisjunction in primary spermatocytes during meiotic metaphase I (MMI)	Miller and Adler, 1992
C-banding	Bone marrow cell micronuclei from male and female Swiss-albino mice	Three mice/sex were dosed with 80 mg/kg HQ by i.p. injection and then killed 24 h later. At least 10 smears were prepared per animal. C-banding was done at least 3 wks after slide preparation. DNA content and area of micronuclei were also determined	In respect to C-banding and DNA content, HQ behaved as a clastogen. However, considering the area measurements, HQ behaved as an aneuploidogen	Van Hummelen et al., 1992
Chromosomal aberration	Male mouse germ cells	Five adult male mice were dosed and one was used as a control for each group. Three experiments were performed; HQ was dissolved in sterile water and administered by a single i.p. injection at a volume of 0.1 ml/10 g body weight *Experiment 1:* analysis of chromosmal aberrations was performed in diakinesis-metaphase I cells 1, 5, 9, 11, 12, and 13 days after dosing with 80 mg/kg HQ *Experiment 2:* two groups of mice were dosed with 40 or 120 mg/kg HQ and testes were sampled 12 days later *Experiment 3:* mice were dosed with 40, 80, or 120 mg/kg HQ and differentiating spermatogonia were analyzed for chromosomal aberrations 24 h later	*Experiment 1:* Due to HQ treatment, it was not possible to analyze structural chromosomal aberrations in cells at diakinesis-metaphase I 24 h after dosing. Significant differences from controls were observed on days 9 and 13. Exchange configurations were only increased at 13 days after treatment *Experiment 2:* At 40 mg/kg, significant increases were observed in the number of chromosomal aberrations. A plateau was reached between 40 and 80 mg/kg. A significant difference in the number of aberrant cells observed after treatment with 120 mg/kg was not significantly different from controls *Experiment 3:* The mitotic index was significantly increased at 40 and 120 mg/kg, but not at 80 mg/kg, when compared with controls. At all doses, the frequency of aberrant cells, excluding gaps, was statistically greater than control values. HQ was clastogenic in male mouse germ cells	Ciranni and Adler, 1991

(continued)

TABLE 2. *Continued*

Test	Organism and strain	Methods	Results and comments	Reference
Chromosomal aberration	Mouse bone marrow cells	HQ in bidistilled water was tested at a dose range of 40–100 mg/kg. Five male and five female mice were used per i.p. dose per interval. The negative control group consisted of one male and one female per dose per interval	Chromosomal aberrations were increased in a dose-dependent manner; both chromatid breaks and exchanges were induced. HQ was clastogenic. A sex difference was not observed	Xu and Adler, 1990
Chromosome malsegregation	Male mouse bone marrow cells	Four mice per group were dosed with an injection of 40, 80, or 120 mg/kg HQ in water. Two h before dosing, mice were given 25 mg of BrdU by s.c. implantation. Bone marrow was removed 18 and 24 h after dosing	After 18 h, HQ significantly increased AGT values at 80 and 120 mg/kg and significantly induced hyperploid cells in the 2N = 41,42 class at 80 mg/kg	Pacchierotti et al., 1991
Chromosomal aberration	Mouse bone marrow cells	HQ was assayed in mouse bone marrow cell cultures	HQ caused a 5–10% increase in SCEs, a 3–5% increase in aneuploidies, and a borderline positive increase in polyploidies	Marrazzini et al., 1991
Chromosomal aberration	Bone marrow cells from male Swiss CD-1 mice	Three mice per group were dosed with 80 mg/kg HQ in bidistilled water orally and by i.p. injection after s.c. injection of 25 mg BrdU	HQ caused a weak increase in structural aberrations. The % aberrant cells was significantly increased with both routes of administration, with the greatest increase caused by i.p. injection; the most aberrant cells were observed 6 h after oral administration and 24 h after i.p. injection	Marrazzini et al., 1992
Chromosomal aberration	Human lymphocyte cultures	Two cultures per dose were exposed to 3–24 μg/ml HQ 24 h after the addition of 9 μg/ml BrdU and harvested after a total of 72 and 96 h	HQ induced polyploidy. Tetraploid cells were induced with a low frequency at both time periods in the dose range of 12–24 μg/ml without a dose–effect relationship; doses > 24 μg/ml produced toxic effects	Sbrana et al., 1992
c-Mitotic effects	Mouse bone marrow cells	HQ was dissolved in bidistilled water and tested at a dose range of 80–150 mg/kg. Five male mice were dosed by i.p. injection and five were used as negative controls	Mitotic index decreased insignificantly with increased doses. Cytotoxicity before mitosis rather than mitosis-arresting activity was observed at 150 mg/kg, but chromatid spreading and shortening was induced. At 100 and 150 mg/kg, metaphases with spread chromosomes were significantly increased. At 150 mg/kg, anaphase frequencies increased significantly and the chromosomes of the metaphase and anaphase cells were partially clumped	Miller and Adler, 1989

c-Mitotic effects	Human lymphocyte cultures	Two cultures per dose were exposed to 3–24 μg/ml HQ 24 h after the addition of 9 μg/ml BrdU and harvested after a total of 72 and 96 h	HQ induced c-mitoses. Partial c-mitoses were observed, with the greatest number seen at 24 μg/ml. Shortening and spreading of chromatids were induced, but the chromosomes were often clustered. Mitotic indices were decreased (sometimes significantly) at all doses	Sbrana et al., 1992
Chromosome loss	Yeast strain *D6*	A dose range of 500–4,000 μg/ml was tested without S9	HQ induced significant increases in white cycloheximidine resistant colonies, indicating chromosome loss	Parry et al., 1990
Chromosomal malsegregation	*Saccharomyces cerevisiae* D61.M	HQ was dissolved in DMSO and tested at a dose range of 0.05–3.0 mg/ml	Negative	Albertini, 1990
In vitro porcine brain tubulin assembly assay	Porcine brain tubulin	A dose range of 0.005–25 m*M* was tested	HQ had no effect on the steady-state level reached at the end of the assembly process, lag-phase, polymerization velocity, or end absorption. Tubulin disassembly was not affected in regard to reversibility of the assembly process. Depolymerization velocity appeared to be enhanced at high concentrations (>10 m*M*)	Brunner et al., 1991
DNA strand breakage	Supercoiled ϕX-174 DNA	HQ-induced DNA strand breakage was studied in the presence and absence of oxygen scavengers	HQ dose dependently caused single- and double-strand breakages. Doses of 10^{-3}–1.1×10^{-4} *M* were needed for complete loss of supercoiled DNA. Scavengers did not protect DNA from HQ-induced breakage	Lewis et al., 1988
DNA strand breakage			Positive	Shimada et al., 1988
DNA strand breakage	Male Sprague–Dawley rat liver cell nuclei	Cell nuclei were prepared from the livers of rats dosed with HQ and from control animals. Single stand breaks (SSB) were determined by the DNA unwinding technique (Ahnstrom and Erixon, 1973) as modified by Walles and Erixon (1984)	HQ caused a significant increase in SSB as compared with control values	Stenius et al., 1989
DNA strand breakage	Purified DNA	A concentration range of 10–1000 μ*M* was tested	HQ caused DNA strand breakage	Maeda et al., 1990
DNA strand breakage	Supercoiled Bluescript plasmid DNA	The ability of oxidation metabolites of HQ, generated by PHS, to induce SSB was examined. The DNA was incubated with PHS in buffer which contained 100 μ*M* HQ	PHS oxidized HQ to metabolite(s) that caused DNA SSB	Schlosser et al., 1990

(continued)

TABLE 2. *Continued*

Test	Organism and strain	Methods	Results and comments	Reference
DNA strand breakage	Human lung carcinoma cell line A 549	Cells were exposed to HQ in the presence and absence of inhibitors. The induction of DNA SSB and the formation of 8-hydroxyde-oxyguanosine (8-OHdG) was determined	HQ, 800 μM, caused a fast depletion of total thiol content in the cell cultures and the amount in cells remained low HQ, 400–800 μM, produced DNA SSB, but the damage did not increase over time HQ, 100 μM, did not induce DNA damage, but 100 μM HQ plus cigarette smoke caused DNA SSB HQ, 800 μM, did not result in significant 8-OHdG formation Catalase had little effect on the number of DNA SSB induced by HQ, but dimethylthiourea and *o*-phenanthroline reduced the number of HQ-induced SSB. An endonuclease activity inhibitor (AT) and an intracellular calcium chelator (BAPTA) also reduced the number of HQ-induced SSB	Lcanderson and Tagesson, 1992
DNA strand breakage	Rat hepatocytes	HQ was tested at ≤0.4 mM. Cells were also exposed to inhibitors	HQ-induced DNA SSB. HQ had a threshold value at low concentrations, after which the DNA damage increased. Exposure of cultures to 0.3 mM HQ produced twice the damage of that observed in the controls. DNA damage increased with longer exposure, with a lag phase of ~20 min. Pretreatment with a Ca^{2+} inhibitor decreased the amount of DNA damaged produced by HQ. DNA damage was increased by post-treatment with a poly(ADP-ribose) polymerase inhibitor	Walles, 1992
DNA strand breakage	Mouse lymphoma cell line L5178YS (LY-S)	HQ was tested at 0.1 mM, the highest non-toxic dose. Four experiments were performed	HQ did not significantly increase the number of SSB	Pellack-Walker and Blumer, 1986
DNA sequencing technique	^{32}P 5′-end-labelled DNA fragments from human c-Ha-*ras*-1 protooncogene	The DNA fragments were incubated with 5 mM HQ in 200 µl of 20 mM Tris-HCl buffer at pH 8.0. DNA damaged was estimated by gel electrophoretic analysis	HQ caused slight DNA damage	Kawanishi et al., 1989

Foci experiment	Male Sprague–Dawley rats	Rats, some of which were pretreated with diethylnitrosamine (DEN), were dosed with 100 or 200 mg HQ	HQ significantly increased the number of foci when the rats were pretreated with DEN. No foci were observed when rats were not pretreated with DEN HQ, single dose of 200 mg/kg, induced lipid peroxidation. HQ had a dose-related effect on hepatic ODC induction. HQ rapidly depleted glutathione (GSH)	Stenius et al., 1989
DNA synthesis	Mouse lymphoma cell line L5178YS (LY-S)	A modification of the DNA synthesis inhibition assay described by Painter (1977) was performed with and without S9. A dose range of 1×10^{-7}–1×10^{-4} *M* was tested. Several standard mutagens of [^{3}H]thymidine incorporation were used	HQ inhibited DNA synthesis by ~65%. The ED_{50} was 1.0×10^{-5} *M* and the highest nontoxic dose was 40 μM	Pellack-Walker et al., 1985
Nuclear DNA synthesis	Bone marrow cells	HQ, ≤24 μM, was tested in cells that were in the DNA synthetic phase of the cell cycle	HQ inhibited nuclear DNA synthesis. The IC_{50} was 11.2 μM	Lee et al., 1989
mtDNA synthesis	Rat liver mitoplasts and rabbit bone marrow mitochondria in vitro	HQ was tested at ≤10 m*M*	≤10 m*M* HQ did not inhibit hepatic mtDNA synthesis, but ≤10 m*M* HQ produced a dose-dependent inhibition of mtDNA synthesis in rabbit bone marrow mitochondria, with 10 m*M* causing 50% inhibition	Schwartz et al., 1986
DNA synthesis	Cell-free DNA synthetic system	The effect of ≤24 μM HQ on DNA synthesis was examined	DNA synthesis was not inhibited	Lee et al., 1989
DNA synthesis	Bladder epithelial cells from male F344 rats	Five rats were fed 0.8% HQ in feed for 4 wks. Before being killed, all rats were given an i.p. injection of BrdU as a marker. Five rats were fed untreated feed and used as a control group	No increase in labeling indices of treated rats was observed	Kurata et al., 1990
mtDNA transcription	Rabbit bone marrow cell mitoplasts	HQ, ≤5.0×10^{-5} *M*, was added to mitoplasts in vitro	HQ inhibited mtDNA transcription	Rushmore et al., 1983
RNA synthesis	Mouse spleen lymphocytes (from male Swiss mice)	HQ was tested at ≤2×10^{-5} *M*	Dose-dependent inhibition was observed. HQ completely inhibited RNA synthesis at concentrations that had essentially no effect on lymphocyte viability	Post et al., 1985
RNA synthesis	Peritoneal macrophages (from male Swiss–Webster mice)	HQ was tested at ≤2×10^{-5} *M*	Dose-dependent inhibition was observed. HQ had no effect on lymphocyte viability at concentrations ≤1×10^{-4} *M*	Post et al., 1986

(continued)

TABLE 2. *Continued*

Test	Organism and strain	Methods	Results and comments	Reference
mRNA synthesis	Rabbit bone marrow nuclei	HQ, 6×10^{-6} *M*, was incubated under conditions specific for RNA polymerase II. HQ was then tested at the concentration range of 10^{-7}–10^{-3} *M*	Positive. HQ inhibited the incorporation of [^{3}H]UTP into mRNA over a 30-min period. The IC_{50} for HQ varied from 4×10^{-6}–1×10^{-5} *M*	Post et al., 1984
8-OHdG formation in DNA	Calf thymus DNA	Calf thymus DNA was incubated with 50–400 μ*M* HQ. The effects of tyrosine and catalase in addition to HQ was also examined	HQ dose dependently caused hydrogen peroxide and 8-OHdG formation. The hydrogen peroxide and 8-OHdG formation decreased with the addition of tyrosine or catalase to the incubation mixture	Leanderson and Tagesson, 1990
DNA adduct formation	HL-60 cells	Cells were treated for 8 h with 100 μ*M* HQ	HQ produced 0.8–2.0 adducts/10^7 nucleotides	Pongracz et al., 1990
DNA adduct formation	HL-60 cells	^{32}P-postlabeling technique was used to determine DNA adduct formation after the addition of 0–500 μ*M* HQ to cell cultures for 0.5–24 h	A single DNA adduct was produced after 8 h of treatment with 500 μ*M* HQ; the relative adduct level was 7.8 ± 0.8 adducts per 10^7 nucleotides. Adduct formation was linear and time dependent for 50–500 μ*M* HQ	Levay et al., 1991
DNA adduct formation	HL-60 cells	^{32}P-postlabeling technique was used to determine DNA adduct formation after the addition of 0–2,000 μ*M* HQ for 1–72 h	A single DNA adduct was produced by treatment with 500 μ*M* HQ; the relative adduct level was 7.8 ± 0.8 adducts per 10^7 nucleotides. DNA adduct formation was time- and concentration-dependent	Levay and Bodell, 1992
DNA adduct formation	HL-60 cells	^{32}P-postlabeling technique was used to determine DNA adduct formation after 50 μ*M* HQ alone and in combination with 100 μ*M* catechol or 50 μ*M* 1,2,4-benzenetriol was added to cell cultures	DNA modification was increased 5-fold with the addition of catechol and 10-fold with the addition of 1,2,4-benzenetriol as compared with HQ alone	Levay and Bodell, 1991
DNA adduct formation	HL-60 cells	^{32}P-postlabeling technique was used to determine DNA adduct formation after the addition of 50 μ*M* HQ in combination with 50–250 μ*M* catechol or 25–100 μ*M* 1,2,4-benzenetriol to cell cultures for 24 h	Combined treatment of HQ and 250 μ*M* catechol increased adduct formation 2.5-fold. Combined treatment of HQ and 50 or 100 μ*M* 1,2,4-benzenetriol increased adduct formation 2.2- and 2.7-fold, respectively	Levay and Bodell, 1992
DNA adduct formation	Calf thymus DNA	^{32}P-postlabeling technique was used to determine DNA adduct formation after purified calf thymus DNA was reacted with 1 mg HQ and 1 mg *p*-benzoquinone overnight	Five adducts were produced; the relative adduct level was 5.5×10^{-5}	Levay et al., 1991

DNA adduct formation	Rat Zymbal glands (in vitro study); female Sprague–Dawley rats (in vivo study)	Nuclease P1-enhanced ^{32}P-postlabeling assay was used to detect DNA adducts. HQ was used in vitro. HQ/phenol were used in vivo; the bone marrow, Zymbal gland, spleen, and liver were examined for adducts	in vitro: HQ produced DNA adducts in vivo: HQ/phenol did not produce DNA adducts	Reddy et al., 1990
DNA binding	Calf thymus DNA	The binding of PHS catalyzed, arachidonate dependent, oxidation metabolite(s) of 100 μM HQ to calf thymus DNA was examined. The results were compared with those obtained using heat-inactivated PHS as well as those in which indomethacin was added to the culture	DNA binding increased and was 65% greater than binding that occurred using heat-inactivated PHS. Indomethacin partially inhibited the binding	Schlosser et al., 1990
Forward mutation assay	L5178Y $tk^{+/-}$ mouse lymphoma cells	HQ in methanol or DMSO was tested at a concentration range of 0.625–10 μg/ml with S9 (1 run) and at a concentration range of 0.625–25 μg/ml without S9 (3 runs). Controls were used	Positive with S9 at 2.5–10 μg/ml. Positive without S9 at 1.25–10 μg/ml (above this dose it was toxic to cells)	McGregor et al., 1988*a*
Forward mutation assay	Mouse lymphoma L5178Y cells	A concentration range of 2.5–8.5 μg/ml HQ was tested without metabolic activation	Positive	McGregor et al., 1988*b*
Micronucleus test	Bone marrow cells from male Swiss CD-1 mice	A modified procedure of Schmid (1975) was used. Five male mice were dosed orally with 200 mg/kg HQ at a volume of 10 ml/kg. Controls were used	HQ significantly increased the number of MnPCEs as compared with controls	Gad-El Karim et al., 1986
Micronucleus test	Bone marrow cells from gravid Swiss CD-1 mice; fetal liver cells	Gravid mice were dosed with 40 mg/kg HQ by gastric intubation on day 13 of gestation. The mice were killed at various time intervals after dosing	A 1- to 3-fold increase in micronuclei of gravid mice was observed. A 3- to 6-fold increase in micronuclei of fetuses was observed	Ciranni et al., 1988*a*
Micronucleus test	Bone marrow cells from male Swiss CD-1 mice	Mice were dosed with 80 mg/kg HQ in sterile distilled water orally or by i.p. injection. The mice were killed at various intervals after dosing	Oral administration produced a weak micronucleus increase. i.p. administration produced an evident micronucleus increase	Ciranni et al., 1988*b*
Micronucleus test	Mouse bone marrow cells		Positive	Shimada et al., 1988
Micronucleus test	V79 Chinese hamster cells	At least five concentrations were tested; the optimal/maximal concentration was 17.5 μM	Positive	Glatt et al., 1989

(continued)

TABLE 2. *Continued*

Test	Organism and strain	Methods	Results and comments	Reference
Micronucleus test	Mouse bone marrow cells	Male and female mice were given a single i.p. dose of 30–100 mg/kg HQ or male mice were given doses of 15 and 75 mg/kg HQ daily for up to 3 days. The mice were killed at various intervals after dosing. Vehicle was bidistilled water and negative controls were used	A dose-dependent, nonlinear, response was observed after a single dose. No sex difference was observed. Additivity of the response was observed for multiple doses, with a significant effect produced after 3 days of dosing with 15 mg/kg. However, the three daily treatments were not as effective as the single total dose (45 mg/kg)	Adler and Kliesch, 1990
Micronucleus test	Peripheral human lymphocytes	The cytokinesis-blocked micronucleus assay was performed. An antikinetochore antibody was used to distinguish micronuclei containing whole chromosomes from those containing acentric fragments. HQ was tested alone, in combination with phenol, and with equimolar concentrations of catechol	Positive. Only minor increases were observed with the addition of phenol. A synergistic effect was observed using equimolar concentrations of HQ and catechol	Robertson et al., 1990
Micronucleus test	Human lymphocytes	A modified micronucleus assay was performed and an antikinetochore was used to distinguish micronuclei that have a high probability of containing a whole chromosome from those containing acentric fragments. A dose range of 2.0–150 μM HQ was used	An 11-fold increase in micronuclei was observed with 125 μM HQ, with a clear dose response being seen from 25–125 μM HQ. Significant increases in kinetochore-positive micronucleated cells were also observed with $\geq$75 μM HQ	Yager et al., 1990
Micronucleus test	Mouse bone marrow cells (male and female)	Mice were dosed with a single i.p. injection of 20–320 mg/kg HQ. The mice were killed at various intervals	Positive. No sex difference was observed. HQ was clastogenic in mouse bone marrow cells	Adler et al., 1991
Micronucleus test	Mouse bone marrow cells	HQ was assayed in mouse bone marrow cultures	HQ increased the number of MNPCEs $\geq$ 10 times that of control values	Marrazzini et al., 1991
Micronucleus test	Male mouse bone marrow cells	Four mice per group were dosed with an injection of 40, 80, or 120 mg/kg HQ in water. Two h before dosing, mice were given 25 mg of BrdU by s.c. implantation. Bone marrow was removed 18 and 24 h after dosing	Positive. At 18 h, the number of MNPCEs was significantly increased by 80 mg/kg HQ. At 24 h, the number of MNPCEs was significantly increased at all three doses. The % PCEs was significantly decreased by 120 mg/kg HQ at 18 and 24 h	Pacchierotti et al., 1991

Micronucleus test	Bone marrow cells from male Swiss CD-1 mice	Three mice per group were dosed with 80 mg/kg HQ in bidistilled water orally and by i.p. injection after s.c. injection of 25 mg BrdU	i.p. injection resulted in more micronuclei than oral administration. The greatest number of micronuclei in PCEs (10-fold the control value) was observed at 18 h and in normochromatic erythrocytes (5-fold the control value) at 42 h	Marrazzini et al., 1992
Micronucleus test	Bone marrow cells from male Swiss CD-1 mice	Mice were dosed with a single i.p. injection of 40–80 mg/kg HQ or an HQ (40–80 mg/kg) and phenol (40–160 mg/kg) mixture	HQ alone gave weakly positive results. A significant dose-dependent increase in micronuclei in PCEs was observed. HQ and phenol coadministration resulted in a >2-fold increase in the number of micronuclei in PCEs compared with HQ alone	Barale et al., 1990
Micronucleus test	Cultured human lymphocytes	HQ in distilled water was tested at a concentration range of 1–20 μg/ml with S9 for various durations and without S9 for 2 h	Without S9, HQ caused a weak induction of micronuclei at a dose range of 5.5–6 μg/ml; 20 μg/ml was toxic. The presence of S9 did not increase the number of micronuclei	Migliore and Nieri, 1991
Micronucleus test	ENR and LEO human diploid fibroblasts	HQ in DMSO was tested at a concentration range of 0.15–8.1 μg/ml. The induction of CREST-positive and CREST-negative micronuclei and the total number of micronuclei was determined	At a dose of 8.1 μg/ml, HQ caused a significant increase in the number of CREST-negative and total micronuclei in LEO human diploid fibroblasts. There was a strong difference in the sensitivity to micronucleus induction between the two human diploid fibroblast strains	Bonatti et al., 1992
In vivo aneuploidy/ micronucleus assay	Bone marrow cells from male CD-1 mice	Three mice/group were dosed with 20–80 mg/kg HQ by i.p. injection. The induction of MNPCEs and whether they were Kc^+ or Kc^- was determined	At a dose of 40 and 80 mg/kg, HQ caused a significant increase in the number of MNPCEs. The number of Kc^+ MNPCEs was significant at all three doses and the number of Kc^- MNPCEs was significant at doses of 20 and 40 mg/kg HQ. HQ was classified as highly aneuploidogenic	Gudi et al., 1992
Mouse spot test	Female C57BL/6JHan mice	The female mice were mated with T-stock males. Females were given an i.p. injection of 110 mg/kg HQ on day 10 of gestation. Animals were examined for spots one to two times/wk for 2–4 wks	Negative. Round gray spots, caused by lack of pigmentation at the base of the hairs, were observed on the backs of dosed females several days after treatment. These spots were not observed on the offspring. However, an increase of white midventral spots, suggesting cellular toxicity, were observed. The frequency of relevant spots was slightly, but not significantly, increased	Gocke et al., 1983

nella typhimurium were negative for mutagenicity by Hydroquinone (Haworth et al., 1983; Glatt et al., 1989), except for one study in which Hydroquinone was reported to be a direct-acting mutagen to the strains tested (Lin and Lee, 1992). In that study, the chlorinated or nitrosated derivatives of Hydroquinone were more mutagenic than Hydroquinone itself; the mutagenicity of Hydroquinone and its chlorinated and nitrosated derivatives was suppressed by the presence of S9. A *Salmonella* mutagenicity test was negative (Sakai et al., 1985) and a fluctuation test using *S. typhimurium* was positive with and negative without metabolic activation (Koike et al., 1988). A test for 6-thioguanine resistance was positive (Glatt et al., 1989). In an assay in which two genetically manipulated strains of *Streptomyces griseus,* one which detects point mutations and the other frame shift mutations, were used to activate promutagenic chemicals and determine the formation of mutagenic metabolites, Hydroquinone did not produce a positive response (Buchholz et al., 1992).

Hydroquinone induced sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells with and without metabolic activation (Galloway et al., 1987), in Chinese hamster Don cells (Shimada et al., 1988), and in Chinese hamster V79 cells (Glatt et al., 1989). Hydroquinone also increased SCEs in human lymphocyte cells, and a synergistic effect was observed when acetaldehyde was administered with Hydroquinone (Knadle, 1985). The number of SCEs induced by Hydroquinone was increased when the cell cultures were treated with dimethyl maleate before Hydroquinone administration.

The induction of chromosomal aberrations due to Hydroquinone was demonstrated in *Aspergillus nidulans* as increased mitogenic segregation (Kappas, 1989; Crebelli et al., 1991) without metabolic activation (Crebelli et al., 1987; Kappas, 1990) and in CHO cells with metabolic activation; in CHO cells without metabolic activation, increases were insignificant (Galloway et al., 1987). Hydroquinone induced mitotic division aberrations in two Chinese hamster cell lines (Parry et al., 1990) and it increased the frequency of mitotic crossovers in *A. nidulans* (Crebelli et al., 1991). Hydroquinone induced aneuploidy in male mouse germ cells (Miller and Adler, 1992) and acted as an aneuploidogen when assayed with micronuclei from mouse bone marrow cells (Van Hummelen et al., 1992). Hydroquinone was clastogenic for male mouse germ cells (Ciranni and Adler, 1991) and for mouse bone marrow cells. Chromosomal aberrations were induced in mouse bone marrow cells (Xu and Adler, 1990; Pacchierotti et al., 1991; Marrazzini et al., 1991; Marrazzini et al., 1992; Sbrana et al., 1992) and c-mitotic effects were observed in mouse bone marrow cells (Miller and Adler, 1989) and in human lymphocyte cultures (Sbrana et al., 1992). Hydroquinone induced chromosome loss in yeast strain *D6* without metabolic activation (Parry et al., 1990), but did not induce chromosomal malsegregations using *Saccharomyces cerevisiae* D61.M (Albertini, 1990).

In an in vitro porcine brain tubulin assembly assay, Hydroquinone had no effect on the assembly of porcine brain tubulin (Brunner et al., 1991). High concentrations of Hydroquinone led to an enhancement of the depolymerization velocity.

Hydroquinone induced DNA strand breaks in a number of studies (Lewis et al., 1988; Shimada et al., 1988; Stenius et al., 1989; Maeda et al., 1990; Schlosser et

al., 1990; Leanderson and Tagesson, 1992; Walles, 1992); however, one study did not find Hydroquinone-induced DNA strand breakage (Pellack-Walker and Blumer, 1986). Slight DNA damage was observed using a sequencing technique (Kawanishi et al., 1989). Enzyme-altered rat liver foci were also observed (Stenius et al., 1989).

Hydroquinone inhibited DNA synthesis with and without metabolic activation (Pellack-Walker et al., 1985), nuclear DNA synthesis (Lee et al., 1989), and mitochondrial DNA (mtDNA) synthesis in rabbit bone marrow mitochondria but not rat liver mitoplasts (Schwartz et al., 1986). However, in a cell-free DNA synthetic system, Hydroquinone did not inhibit DNA synthesis (Lee et al., 1989); in another study, a significant increase in DNA synthesis in rat bladder epithelium cells was not observed (Kurata et al., 1990). (mtDNA) transcription synthesis was inhibited (Rushmore et al., 1983), as was RNA synthesis (Post et al., 1985, 1986) and mRNA synthesis under conditions specific for RNA polymerase II (Post et al., 1984).

Hydroquinone caused the dose-dependent formation of hydrogen peroxide and 8-hydroxydeoxyguanosine (8-OHdG) in calf thymus DNA (Leanderson and Tagesson, 1990).

Treatment of HL-60 cells with Hydroquinone produced DNA adducts (Pongracz et al., 1990; Levay et al., 1991; Levay and Bodell, 1992); the number of adducts formed was significantly increased with the addition of catechol or 1,2,4-benzenetriol (Levay and Bodell, 1991; Levay and Bodell, 1992). Adducts also formed by treating calf thymus DNA with a *p*-benzoquinone/Hydroquinone mixture (Levay et al., 1991). Reddy et al. (1990) found that Hydroquinone produced DNA adducts in vitro, but phenol/Hydroquinone did not produce adducts in vivo. The oxidative metabolism of Hydroquinone resulted in the formation of reactive product(s) that irreversibly bound to DNA (Schlosser et al., 1990).

Forward mutation assays with (McGregor et al., 1988*a*) and without (McGregor et al., 1988*a*, 1988*b*) metabolic activation were positive.

Positive micronucleus test results for Hydroquinone were obtained by Gad-El Karim et al. (1986), Ciranni et al. (1988*a*,*b*), Shimada et al. (1988), Glatt et al. (1989), Adler and Kliesch (1990), Robertson et al. (1990), Yager et al. (1990), Adler et al. (1991), Marrazzini et al. (1991), Pacchierotti et al. (1991), and Marrazzini et al. (1992). In the study by Robertson et al. (1990), coadministration of equimolar concentrations of Hydroquinone and catechol produced a significant synergistic effect on micronucleated cells as compared with Hydroquinone alone. In micronucleus tests reported by Barale et al. (1990) and Migliore and Nieri (1991), the results were weakly positive. A combined dose of Hydroquinone and phenol gave positive results. In one study, Hydroquinone caused a significant increase in CREST-negative and total micronuclei (Bonatti et al., 1992) and in another, it caused significant increases in kinetochore positive (Kc^+), kinetochore negative (Kc^-), and total micronucleated polychromatic erythrocytes (MNPCEs) (Gudi et al., 1992).

A mouse spot test for somatic gene mutations using Hydroquinone was negative (Gocke et al., 1983).

CARCINOGENICITY

The carcinogenicity studies reviewed in this section are summarized in Table 3.

F344N rats, 65/sex/group, and B6C3F$_1$ mice, 64 or 65 males and 65 females/group, were dosed by gavage with Hydroquinone in deionized water 5 days/week for up to 103 weeks (NTP, 1989; Kari et al., 1992). The rats were dosed with 25 or 50 mg/kg and the mice were dosed with 50 or 100 mg/kg Hydroquinone; a group of rats and a group of mice were dosed with vehicle only and served as controls. Doses were selected based on the results of the subchronic study (NTP, 1989; Kari et al., 1992) summarized previously in this report.

All animals were observed twice daily and clinical signs were recorded monthly. Body weights were measured weekly for 13 weeks, and then monthly. Gross observations were made for all animals at necropsy. Microscopic examinations were performed on selected tissues from all rats, all mice of the control and high-dose groups, and all mice of the low-dose group that died on study. The microscopic examinations of tissues from the mice were performed according to an "inverse pyramid" design (McConnell, 1983*a,b*). After 15 months of dosing, 10 animals/group were randomly selected for interim necropsy and evaluation of clinical chemistry and hematologic parameters.

After 15 months, relative kidney weights of the male rats of the high-dose group were significantly greater than control values. A dose-related increase in the severity of nephropathy was observed in male rats. In female rats, there was a decrease in the occurrence of hyperplasia and neoplasms of the pituitary gland when compared with controls. For female rats of the high-dose group, hematocrit, hemoglobin concentration, and erythrocyte count were decreased when compared with control values. The alkaline phosphatase value was decreased in male rats of the high-dose group and increased in female rats of the low-dose group.

For male mice of the high-dose group, significant increases were observed in hematocrit, erythrocyte count, serum albumin concentration, total protein concentration, and serum alkaline phosphatase and sorbitol dehydrogenase activity after 15 months. For female mice of the high-dose group, serum albumin and total protein concentration were significantly increased and alanine aminotransferase and sorbitol dehydrogenase activity were significantly decreased when compared with the controls. The values for relative liver weight for all mice of the high-dose group, relative kidney weight for all treated female mice, and relative brain weight for female mice of the high-dose group were significantly increased compared with control values. Dose-related hepatic lesions, centrilobular fatty changes, and cytomegaly were observed in treated male mice. Hepatocellular neoplasms were observed in male and female mice; the number of neoplasms observed was not significantly greater than in the controls and therefore not attributed to the administration of Hydroquinone.

At study termination, there was no significant difference in the mortality rate between the dosed and control male rats; however, the number of moribund animals killed was greater for dosed male rats than controls after week 90. Body weights of male rats of the high-dose group were decreased after week 73 and of male rats of the low-dose group after week 89 when compared with the controls.

The relative brain, kidney, and liver weights of the male rats of the high-dose group were significantly increased when compared with control values.

The severity of spontaneous nephropathy for male rats of the high-dose group and papillary hyperplasia of the transitional epithelium overlying cysts of the renal papillae in all male rats was increased when compared with the controls. Renal tubular adenomas occurred in treated males but not in control males, and the incidence in the high-dose group was significantly different from controls. Tubular hyperplasia was observed in two male rats of the high-dose group. There were significantly fewer adenomas and adenomas and carcinomas (combined) of the anterior pituitary gland in male rats of the high-dose group than the controls; tumor incidence at this site was not considered to be dose related.

There was no difference in mortality between treated and control female rats. Body weights of all female rats remained similar throughout the study. A significant increase in the incidence of mononuclear cell leukemia was observed in treated female rats compared with the controls. Stage 3 leukemia, in which there is marked effacement of the spleen and advanced infiltration of the liver or other organs with neoplastic cells, was observed in 14 female rats of the high-dose group and eight female rats of the low-dose group as compared with five controls. c-Cell adenoma and carcinoma (combined) occurrence was significantly decreased in females of the low-dose group.

For rats, the authors concluded "there was evidence of Hydroquinone-related carcinogenicity in male F344/N rats, indicated by an increased incidence in tubular cell adenomas of the kidney and in female rats, as shown by an increase in mononuclear cell leukemia."

There was no difference in mortality between treated and control male mice. Body weights of male mice of the high-dose group were decreased after week 93 as compared with control values. The relative liver weights of treated male mice were increased compared with control values. In male mice, dose-related non-neoplastic hepatic lesions were observed. There was no significant difference in the occurrence of hepatic adenomas and carcinomas (combined) between test and control mice. Follicular cell hyperplasia of the thyroid gland was increased in treated mice.

There was no difference in mortality between treated and control female mice. Body weights of female mice of the high-dose group were decreased after week 20 compared with control values. Relative liver weights of females of the high-dose group were increased compared with controls. There was a significant increase in the number of hepatocellular adenomas in treated female mice. An increase in follicular cell hyperplasia of the thyroid gland was observed in dosed females.

For mice dosed with Hydroquinone, the authors concluded "there was no evidence in male $B6C3F_1$ mice, and there was evidence of carcinogenicity in female mice based on increases in hepatocellular neoplasms, mainly adenomas. Administration of Hydroquinone was also associated with thyroid follicular cell hyperplasia in both male and female mice and anisokaryosis, multinucleated hepatocytes, and basophilic foci of the liver in male mice."

Hydroquinone was fed to F344 rats and $B6C3F_1$ mice in the diet to evaluate its carcinogenic potential (Shibata et al., 1991). Rats and mice, 30 male and 30 female

TABLE 3. *Hydroquinone (HQ) carcinogenicity studies*

Number, species and sex	Dose	Route of administration	Methods	Results and comments	Reference
F344N rats 65/sex/gp	25 or 50 mg/kg	Orally by gavage	Rats were dosed 5 days/wk for up to 103 wks. One group was dosed with vehicle (deionized water) and served as a control group	*Males:* Some evidence of carcinogenic activity of HQ as shown by marked increased incidence in renal tubular cell adenomas *Females:* Some evidence of carcinogenic activity of HQ as indicated by increased incidence of mononuclear cell leukemia	NTP, 1989
$B6C3F_1$ mice 64 or 65 males and 65 females/gp	50 or 100 mg/kg	Orally by gavage	Mice were dosed 5 days/wk for up to 103 wks. One group was dosed with vehicle (deionized water) and served as a control group	*Males:* No evidence of carcinogenic activity of HQ *Females:* Some evidence of carcinogenic activity of HQ as shown by increases in hepatocellular neoplasms, mainly adenomas	NTP, 1989
F344 rats 30/sex/gp	0.8% HQ	Orally in feed	Rats were fed treated diet for 104 wks. A control group was fed untreated feed	*Males:* A statistically significant number of renal tubular hyperplasias and microscopic adenomas developed *Males and females:* The average number of foci/cm^2 in analyzed liver sections was significantly decreased. There were no dose-related proliferative or neoplastic lesions nor any other microscopic lesions observed in the nonglandular or glandular stomach HQ has apparent carcinogenic potential for rodents; therefore it may play a role in human cancer development	Shibata et al., 1991
$B6C3F_1$ mice 30/sex/gp	0.8% HQ	Orally in feed	Mice were fed untreated diet for 96 wks. A control group was fed untreated feed	*Males:* The significant development of renal tubular hyperplasia and a nonstatistically significant increase in renal cell adenomas was observed. The incidence of hepatocellular adenomas, but not hepatocellular carcinomas, was increased. The occurrence of hepatic foci and the number of foci/cm^2 was significantly increased *Males and females:* The occurrence of squamous cell hyperplasia, but not tumor development, in the nonglandular epithelium was significantly increased HQ has apparent carcinogenic potential for rodents; therefore it may play a role in human cancer development	Shibata et al., 1991
Male Syrian golden hamsters 15/gp	0.5% HQ	Orally in feed	Hamsters were fed treated feed for 20 wks. A control group was fed untreated feed	There was no significant difference between treated and control animals in the incidence of mild or moderate hyperplasia of the nonglandular epithelium of the stomach. HQ had no structure-related activity in inducing proliferative neoplasms in the nonglandular portions of the stomach	Hirose et al., 1986
Male F344 rats 15 treated and 10 control animals	0.8% HQ	Orally in feed	The respective groups were fed treated or control diet for 51 wks	There were no significant differences observed between treated and control animals in the occurrence of lesions of the nonglandular or glandular stomach	Hirose et al., 1989

Male F344 rats 15 treated and 10 control animals	0.8% HQ	Orally in feed	The respective groups were fed treated or control diet for 49 wks	Squamous hyperplasia was increased for treated rats (26.7%) as compared with controls (0%)	Yamaguchi et al., 1989
Male F344 rats 5/gp	0.8% HQ	Orally in feed	Rats were fed treated feed for 8 wks. A control group was fed untreated feed. All animals were given an i.p. injection of BrdU 1 h before being killed	There was no significant difference between treated and control rats in cell proliferation in the nonglandular gastric epithelium or the pyloric glandular epithelium	Shibata et al., 1990
			Tumor promotion		
Male F344 rats 20/gp	0.8% HQ	Orally in feed	Two groups of rats were given 150 mg/kg MNNG by stomach tube. Starting 1 wk later, the rats were fed treated or untreated feed for 51 wks	Larger neoplasms were observed in the nonglandular epithelia of HQ-treated rats compared with controls. However, there was no difference in the incidence of nonglandular or glandular stomach lesions between the groups	Hirose et al., 1989
Male F344 rats 15/gp	0.8% HQ	Orally in feed	Two groups of rats were given three i.p. injections of 25 mg/kg MNAN at wks 0, 1, and 2. Starting 1 wk later, rats were fed treated or untreated feed for 49 wks	A nonsignificant increase in the development of squamous cell carcinomas, including early in situ lesions, was observed in the esophagus of test animals compared with controls, but quantitative analysis reported that the number of squamous cell carcinomas was significantly greater in treated rats. The incidence of pulmonary alveolar cell hyperplasia was significantly decreased for treated animals. No significant difference was observed between treated and control rats in the incidence of nonglandular hyperplasia. HQ was marginally effective in enhancing esophageal carcinogenesis and demonstrated marginal activity in the promotion of upper digestive tract carcinogenesis	Yamaguchi et al., 1989
Male F344 rats 20 pretreated/gp; 10 nonpretreated	0.8% HQ	Orally in feed	Two groups were given 0.05% BBN in drinking water for 4 wks. Starting 3 days later, the rats were fed treated or untreated feed until the end of wk 36. A third, nonpretreated, group was fed treated feed (starting at the same time as the pretreated animals).	No differences in the incidence or number of PN hyperplasia, papilloma, or carcinoma were observed between the BBN-pretreated and nonpretreated test groups and the pretreated controls	Kurata et al., 1990
Male F344 rats 20 pretreated/gp; 10 nonpretreated	0.8% HQ	Orally in feed	Two groups were given 0.1% DHPN in drinking water for 2 wks. The pretreated and nonpretreated groups were then fed treated or untreated feed for 30 wks	Localized dark or whitish nodular hyperplastic and/or neoplastic lesions were observed in the lungs of pretreated test animals. No differences were observed between pretreated test and control rats in the incidences of pulmonary adenoma, carcinoma, or combined tumors, nor in the number of lesions observed in the thyroid gland, urinary bladder, or kidneys. Squamous cell carcinomas of the nonglandular stomach were observed in one pretreated test rat. Pulmonary, thyroid gland, urinary bladder, and/or kidney neoplastic lesions were not observed for nonpretreated HQ-dosed rats	Hasegawa et al., 1990

per species, were fed a diet containing 0.8% Hydroquinone for 104 and 96 weeks, respectively. The same number of male and female rats and mice was fed untreated diet and served as control groups. All animals were examined twice daily. Body weights were measured weekly throughout week 14, and then once every 4 weeks. Feed and water intake were determined over a 2-day period before each weighing. All surviving animals were fasted and killed at the termination of dosing. Necropsy was performed and the liver and kidneys of all animals were weighed.

No dose-related changes were observed for either species during the study, and there was no significant difference in survival for treated animals compared with controls. Body weight gains of dosed male and female rats and male mice were significantly reduced compared with the controls. Feed and water consumption were comparable for treated and control animals. The average amount of Hydroquinone intake, based on feed consumption, was 351 and 368 mg/kg/day for male and female rats, respectively, and 1,046 and 1,486 mg/kg/day for male and female mice, respectively.

There was a significant increase in the absolute and relative liver and kidney weights of dosed male rats and the relative kidney weights of dosed female rats as compared with controls. In mice, the relative liver and kidney weights of dosed females were significantly increased compared with the controls.

At necropsy, granular appearance or indentation of the kidneys was increased in treated male rats. Small nodular lesions were visible in the mucosa of the nonglandular stomach of many dosed male and female mice.

Chronic nephropathy was observed in treated and control male rats upon microscopic examination; however, it was more severe in the dosed males. Pelvic epithelial hyperplasia, a component of advanced chronic nephropathy, was increased in treated male rats. Slight chronic nephropathy was observed in female rats fed Hydroquinone. Statistically significant numbers of renal tubular hyperplasia, for which cystic forms were frequent and terms such as "dysplastic foci" and "dysplastic tubular epithelium" were considered synonymous, and microscopic adenomas, cystic or solid forms, developed in male rats fed Hydroquinone. The average number of foci per cm^2 of analyzed liver sections was significantly decreased for male and female rats fed Hydroquinone. Most of the hepatic foci were basophilic. There were no dose-associated proliferative or neoplastic lesions observed in the nonglandular or glandular stomach of rats, nor were any other dose-related microscopic lesions observed.

In male mice, microscopic lesions included significant numbers of renal tubular hyperplasia and an increase that was not statistically significant in renal cell adenomas. The characteristics of the renal lesions were similar to those observed in treated rats. Also in dosed male mice, a statistically significant increase in centrilobular hypertrophy of hepatocytes was observed. The number of hepatocellular adenomas, but not of hepatocellular carcinomas, was increased in male mice fed Hydroquinone compared with the controls. The occurrence of hepatic foci of the basophilic, eosinophilic, and clear cell types and the numbers of foci per cm^2 of liver were significantly increased for dosed male mice. In treated male and female mice, the occurrence of squamous cell hyperplasia, but not tumor

development, in the nonglandular gastric epithelium was significantly increased. There was no significant difference in tumor incidence for any other organ between treated and control animals.

The authors concluded the "study strongly suggested that since Hydroquinone has apparent carcinogenic potential for rodents, there is a possibility that it may play a role in human cancer development."

Fifteen male Syrian golden hamsters were given 0.5% Hydroquinone in the feed for 20 weeks to determine whether Hydroquinone would induce lesions in the squamous portion of the stomach and its effect on proliferation of the glandular stomach and urinary bladder (Hirose et al., 1986). A group of 15 hamsters was fed untreated feed and served as the control group. At the termination of dosing, the animals were killed and underwent necropsy, and nonglandular and glandular portions of the stomach and sections of the urinary bladder were removed for microscopic examination.

There was no significant difference in terminal body weights between the test and control groups. The liver weights of treated animals were greater than those of the controls. The number of animals with mild or moderate hyperplasia of the nonglandular gastric epithelium was not significantly different from the controls. No abnormal observations were made in any examined organs. Upon autoradiographic examination, the labeling indices in the nonglandular portion of the stomach, pyloric region of the glandular portion of the stomach, and the urinary bladder increased insignificantly when compared with control values. Hydroquinone had no structure-related activity in inducing proliferative neoplasms in the nonglandular portion of the stomach.

In order to examine the carcinogenic potential of Hydroquinone on the nonglandular and glandular portions of the stomach, 15 male F344 rats were fed a diet containing 0.8% Hydroquinone (Hirose et al., 1989). Ten male F344 rats ate untreated feed for 51 weeks and served as controls. Body weights and feed consumption were measured every 2–4 weeks. At study termination, all surviving animals were killed and underwent necropsy, and sections from the nonglandular and glandular stomach were removed for microscopic examination.

All rats survived until study termination. The terminal body weights were decreased and the relative kidney weights were significantly increased for the test animals compared with the controls. There was no significant difference in the incidences of gastric lesions (squamous portion), i.e., hyperplasia, papilloma, carcinoma in situ, and squamous cell carcinomas, between the test and control group. Also, there were no significant differences between the treated and control group in the incidences of glandular stomach lesions, i.e., adenomatous hyperplasia or adenocarcinoma in the fundic or pyloric regions.

Male F344 rats were used to examine the carcinogenic potential of Hydroquinone on the upper gastrointestinal tract (Yamaguchi et al., 1989). Fifteen rats were fed a diet containing 0.8% Hydroquinone for 49 weeks while 10 rats were fed untreated feed and served as controls. Body weights and feed consumption were measured every 2–4 weeks. All surviving animals were killed and underwent necropsy at study termination, and portions of the upper gastrointestinal tract were removed for microscopic examination.

All test and control rats survived until study termination. Terminal body weights were significantly decreased and relative kidney weights were significantly increased for test animals as compared with controls. Hyperplasia of the nonglandular portion of the stomach was increased for treated rats (26.7%) as compared with controls (0%).

Five male F344 rats were fed 0.8% Hydroquinone in the diet for 8 weeks to determine the carcinogenic potential on the nonglandular and glandular portions of the stomach; a control group of five rats was fed untreated feed (Shibata et al., 1990). At the end of 8 weeks, each rat was given an i.p. injection of bromodeoxyuridine and killed 1 h later. No rats died on study. No significant differences in body weight or feed consumption were found between the treated and control groups. Cell proliferation in the nonglandular gastric and the pyloric glandular epithelium were similar for the treated and control groups.

TUMOR PROMOTION

Male F344 rats were used to examine the effects of Hydroquinone on the nonglandular and glandular portions of the stomach after *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) administration (Hirose et al., 1989). Two groups of 20 rats were dosed with 150 mg/kg MNNG by stomach tube and then, starting 1 week later, were fed either 0.8% Hydroquinone in feed or untreated feed for 51 weeks. The group given untreated feed served as MNNG-pretreated controls. Body weights and feed consumption were measured every 2–4 weeks. At the termination of dosing, all surviving animals were killed and underwent necropsy; sections of the nonglandular and glandular stomach were removed for microscopic examination.

Fifteen pretreated controls and 16 pretreated test animals survived until study termination. Terminal body weights of the test animals were decreased as compared with controls. The relative liver and kidney weights of MNNG-pretreated rats fed Hydroquinone were greater than the control values. The neoplasms observed in the squamous epithelia of the nonglandular stomach of rats dosed with Hydroquinone after MNNG pretreatment were larger than those observed for pretreated control rats. However, there was no significant difference in the incidence of squamous epithelium lesions, i.e., hyperplasia, papilloma, carcinoma in situ, and squamous cell carcinomas, between the test and control group. Also, there were no significant differences between the treated and control group in the incidence of glandular stomach lesions, i.e., adenomatous hyperplasia or adenocarcinoma of the fundic or pyloric regions.

Male F344 rats, 15 per group, were given three i.p. injections of 25 mg/kg methyl-*N*-amylnitrosamine (MNAN) at weeks 0, 1, and 2 of the study and then, starting 1 week later, were fed a diet containing 0.8% Hydroquinone or untreated feed for 49 weeks (Yamaguchi et al., 1989). Body weights and feed consumption were measured every 2–4 weeks. At the end of week 52 of the study, all surviving animals were killed and underwent necropsy; portions of the upper gastrointestinal tract were removed for microscopic examination.

Twelve pretreated test and 11 pretreated control animals survived until study

termination. Terminal body weights were significantly less and relative kidney weights were significantly greater for the test animals as compared with controls. In the esophagus, a nonsignificant increase in the development of squamous cell carcinomas, including early in situ lesions, was observed for test animals as compared with the controls; but using quantitative analysis, the number of squamous cell carcinomas was significantly greater in treated rats compared with controls. The incidence of pulmonary alveolar cell hyperplasia was significantly decreased for animals fed Hydroquinone as compared to MNAN-pretreated controls. There was no significant difference in the incidence of hyperplasia of the nonglandular mucosa in treated rats. Hydroquinone was marginally effective in enhancing esophageal carcinogenesis and demonstrated marginal activity in the promotion of upper digestive tract carcinogenesis.

Twenty male F344 rats were given 0.05% *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) in drinking water for 4 weeks and then, starting 3 days later, were fed a diet containing 0.8% Hydroquinone until the end of week 36 (Kurata et al., 1990). A control group of 20 rats was dosed with BBN but maintained on untreated feed. A third group of 10 rats was fed 0.8% Hydroquinone in the diet without BBN pretreatment. Dosing for nonpretreated rats started 4 weeks and 3 days after study initiation. After 36 weeks, all surviving animals were killed and various tissues were removed for microscopic examination.

All pretreated and nonpretreated test animals and 19 control animals survived until study termination. Terminal body weights were significantly decreased for BBN-pretreated test animals as compared with controls; no difference was observed for nonpretreated animals dosed with Hydroquinone. There were no differences observed in the incidence or number of papillary or nodular (PN) hyperplasia, papilloma, or carcinoma between the BBN-pretreated and nonpretreated test groups and the control group.

To evaluate the modifying effects on pulmonary tumorigenesis initiated by *N*-bis(2-hydroxypropyl)nitrosamine (DHPN), 20 male F344 rats were given 0.1% DHPN in drinking water for 2 weeks and then fed diet containing 0.8% Hydroquinone for 30 weeks (Hasegawa et al., 1990). A control group of 20 rats was dosed with DHPN and maintained on untreated feed. A third group of 10 rats was fed diet containing 0.8% Hydroquinone without DHPN pretreatment for 30 weeks. Body weights and feed consumption were measured periodically. After 32 weeks, all surviving animals were killed and the lungs, thoracic tissues, and various other tissues were removed for microscopic examination.

All rats survived until study termination. Terminal body weights were significantly decreased and absolute lung weights were significantly increased for DHPN-pretreated test animals as compared with nonpretreated test animals but not as compared with DHPN-pretreated control animals. Absolute liver weights for DHPN-pretreated test rats were significantly increased compared with DHPN-pretreated controls and significantly decreased compared to nonpretreated test rats. Localized dark or whitish nodular lesions, which were microscopically hyperplastic and/or neoplastic, were observed in the lungs of DHPN-pretreated test animals.

No differences were observed in the incidences of pulmonary adenoma, carci-

noma, or combined tumors between the DHPN-pretreated test and control groups, nor were there differences observed in the number of lesions in the thyroid glands, urinary bladder, or kidneys. Squamous cell carcinomas of the nonglandular stomach were observed in one DHPN-pretreated rat dosed with Hydroquinone. Pulmonary, thyroid gland, urinary bladder, and/or renal neoplastic lesions were not observed in the rats of the nonpretreated test group.

CLINICAL ASSESSMENT OF SAFETY

Sensitization

Eighty patients positive to at least one hapten of the *para* group of the ICDRG standard series (*p*-phenylenediamine (PPD), diaminodiphenylmethane, benzocaine, or PPD mix) were tested using the A1 test (reference and/or test description not given) with 50 μl of a prepared solution of 0.5% Hydroquinone (Picardo et al., 1990*b*). No reactions to Hydroquinone were observed.

Dermal Effects

Dermatitis has been caused by contact with Hydroquinone and from application of an "antiseptic oil" which contained a trace amount of Hydroquinone (Deichmann, 1983).

Hydroquinone has caused hypomelanosis–hyperpigmentation of the skin and depigmentation of black skin (Deichmann, 1983).

Ocular Effects

Exposure to Hydroquinone in vapor or dust at concentrations of 10–30 mg/m^3 air has resulted in keratitis, discoloration of the conjunctiva, and corneal changes (Deichmann, 1983). The corneal changes, especially alteration of the curvature, remained after stain and pigment disappeared.

Toxic Effects

Ingestion of 1 g Hydroquinone by an adult produced tinnitus, nausea, dizziness, a sense of suffocation, increased respiration rate, vomiting, pallor, muscular twitching, headache, dyspnea, cyanosis, delirium, green to brownish-green urine, and collapse (Deichmann, 1983). Ingestion of 5–12 g Hydroquinone has resulted in death, apparently due to respiratory failure and anoxia.

EPIDEMIOLOGY

Between 35 and 45% of American women dye their hair, often at monthly intervals, over a period of years (CTFA, 1993). This estimate is drawn from market research data on hair dye product use, generally from females aged 15 to 60 years.

A number of epidemiologic studies have investigated the association between cancer and occupation as a hairdresser or barber, or between cancer and personal use of hair dyes. The World Health Organization's International Agency for Re-

search on Cancer (IARC) empaneled a Working Group on the Evaluation of Carcinogenic Risks to Humans to review all available data on these issues. The Working Group met October 6–13, 1992, in Lyon, France (IARC, 1993).

The charge to the IARC Working Group was to ascertain whether all appropriate data had been collected and were being reviewed; to evaluate the results of the epidemiologic and experimental studies and prepare accurate summaries of the data; and to make an overall evaluation of the carcinogenicity of exposure to humans.

The IARC Working Group conclusions were: "There is *limited evidence* that occupation as a hairdresser or barber entails exposures that are carcinogenic." Hence: "Occupation as a hairdresser or barber entails exposures that *are probably carcinogenic* (*Group 2A*)." And: "There is *inadequate evidence* that personal use of hair colourants entails exposures that are carcinogenic." Hence: "Personal use of hair colourants *cannot be evaluated as to its carcinogenicity* (*Group 3*)." (IARC, 1993).

SUMMARY

This Addendum to the Final Report on Hydroquinone was prepared in response to the release of an NTP (1989) report of an oral carcinogenicity study. In the original CIR report, it was concluded that Hydroquinone was safe for cosmetic use at ≤1% in formulations designed for discontinuous, brief use followed by rinsing from skin and hair. This conclusion applied primarily to the use of Hydroquinone in hair dye formulations. The use of Hydroquinone to lighten the skin was not addressed because such use is regarded by FDA as a drug use.

In 1993, Hydroquinone was reported to be used in 206 formulations, 185 hair dyes, two lipsticks, one skin freshener, and 18 other skin care preparations.

Hydroquinone in an alcoholic vehicle was absorbed through the skin of the forehead of male subjects; absorption of Hydroquinone from a solution that also contained Escalol 507 (a sunscreen) and Azone (a penetration enhancer) was 35 ± 17%, from a solution containing Azone was 66 ± 13%, from a solution containing Escalol 507 was 26 ± 14%, and from a solution containing only Hydroquinone was 57 ± 11%. The average percutaneous absorption rate of Hydroquinone using 48-h excretion data from dermal and i.v. absorption studies using dogs was estimated to be ~0.15 nmol/cm^2/min (1.1 μg/cm^2/h). Hydroquinone was rapidly absorbed and excreted by male and female Fischer rats following oral administration; overall recovery was ≥96% from females after 24 h and from males after 48 h. In a study using urinary excretion data, dermal absorption was estimated to be 10.5% for male rats using 72-h data and 11.5% for female rats using cumulative 48-h data.

Hydroquinone was found to have some immunologic effects; it especially had effects on bone marrow. In an FOB, Hydroquinone was not found to cause central or peripheral nervous system lesions. Hydroquinone was nephrotoxic in male F344 rats. Hydroquinone also showed cytotoxic properties.

According to the terminology of Hodge and Sterner (1949), Hydroquinone is slightly toxic, with an oral LD_{50} of 743 and 627 mg/kg for male and female rats, respectively.

Administration of Hydroquinone to rats in drinking water (2,500–10,000 ppm) for 8 weeks resulted in significant increases in liver and kidney weights. Hydroquinone administered orally to rats (63–1,000 mg/kg) and mice (31–500 mg/kg) for 14 days resulted in tremors and deaths in the high-dose groups. Dermal administration to rats (240–3,840 mg/kg) and mice (300–4,800 mg/kg) for 14 days caused neither death nor any significant adverse effects. For mice given i.p. injections of 10 mg/kg Hydroquinone for 6 weeks, it was concluded that Hydroquinone may cause hematologic injury.

Rats given 1,000–4,000 ppm Hydroquinone in drinking water for 15 weeks had significantly increased liver and kidney weights. Oral administration of 25–400 mg/kg Hydroquinone to rats and mice for 13 weeks resulted in mortality in the high-dose groups for both rats and mice. Other adverse signs, such as lethargy, tremors, and changes in relative liver to body weight ratios, were observed.

Dermal application of 25 or 150 mg/kg Hydroquinone to rats produced slight to severe erythema.

In a Magnusson–Kligman guinea pig maximization test, Hydroquinone was classified as an extreme sensitizer. Hydroquinone was positive for sensitization in an LLNA.

Oral administration of Hydroquinone did not produce embryotoxic, fetotoxic, or teratogenic effects in rats, nor did it produce significant adverse reproductive effects in a two-generation study. Using rabbits, various teratogenic/reproductive treatment–related effects were observed at doses of 200–500 mg/kg. All dams dosed with 300–500 mg/kg Hydroquinone died. Some maternal toxicity was observed at a number of dose concentrations.

Hydroquinone induced SCEs, chromosomal aberrations, and mitotic division aberrations increased the frequency of mitotic crossovers, caused c-mitotic effects, and induced chromosome loss. It was clastogenic for male mouse germ cells and for mouse bone marrow cells. Hydroquinone induced DNA strand breaks and inhibited DNA, nuclear DNA, and mtDNA synthesis in rabbit bone marrow mitochondria. It also inhibited mtDNA transcription synthesis and RNA synthesis. Hydroquinone caused the formation of hydrogen peroxide and 8-OHdG in calf thymus DNA and produced DNA adducts in HL-60 and other cells. Forward mutation assays with and without metabolic activation were positive, as were numerous micronucleus assays. Results of the Ames test and a mouse spot test for somatic gene mutations were negative.

In an NTP study, Hydroquinone was given to rats orally by gavage five times per week for up to 103 weeks at doses of 25 or 50 mg/kg. The higher dose induced a significant incidence of renal adenomas in males and both doses caused a significant increase in the incidence of mononuclear cell leukemia in females. Mice were dosed with 50 or 100 mg/kg Hydroquinone following the same schedule as that used for the rats. The incidence of hepatocellular adenoma was significantly increased in female mice.

NTP concluded that Hydroquinone produced "some evidence of carcinogenic activity" for male and female F344/N rats and female $B6C3F_1$ mice but "no evidence of carcinogenic activity" for male $B6C3F_1$ mice in an oral carcinogenicity study.

Shibata et al. (1991) conducted a study in which rats and mice were fed diet containing 0.8% Hydroquinone for 104 and 96 weeks, respectively, and concluded that "the study strongly suggested that since Hydroquinone has apparent carcinogenic potential for rodents, there is a possibility that it may play a role in human cancer development." Hydroquinone did not induce a significant number of neoplasms in either the glandular or nonglandular stomach of hamsters fed 0.5% Hydroquinone in the diet for 20 weeks or rats fed 0.8% Hydroquinone in the diet for 51, 49, or 8 weeks.

When Hydroquinone was fed to rats after pretreatment with MNAN, Hydroquinone was marginally effective in enhancing esophageal carcinogenesis and had marginal activity in the promotion of upper digestive tract carcinogenesis. Other studies did not prove Hydroquinone to be a tumor promoter.

No reaction to Hydroquinone was observed when patients positive to at least one hapten of the *para* group of the ICDRG standard series were tested using the A1 test. Hydroquinone contact has caused dermatitis and Hydroquinone exposure can result in ocular effects. Hydroquinone has caused hypomelanosis–hyperpigmentation of the skin and depigmentation of black skin. Ingestion of 1 g Hydroquinone by humans can produce severe toxicity; ingestion of 5–10 g can be fatal.

DISCUSSION

The CIR Expert Panel previously determined that Hydroquinone was safe for cosmetic use at ≤1% in formulations designed for discontinuous, brief use followed by rinsing from the skin and hair. The conclusion was reached primarily for Hydroquinone's use in hair dyes because at the time the conclusion was made, the only confirmed use of Hydroquinone in cosmetic products was in hair dye formulations. The use of Hydroquinone in skin preparations as a skin lightener was regarded as a drug use.

Following the November 30–December 1, 1992 Expert Panel meeting, at which the safety of use of Hydroquinone was discussed, FDA advised CIR that in 1993, Hydroquinone has been reported to be used in lipstick formulations, skin lotions, and other nonrinse-off cosmetic products that may not necessarily be classified as drugs. Therefore, in accordance with the CIR Procedures, the conclusion on the safety of the use of Hydroquinone in cosmetic products must address both types of products, i.e., rinse-off and leave-on products.

Since the release of the initial final report in 1986, additional data have become available. A 1989 NTP oral carcinogenicity study using rats and mice indicated that Hydroquinone showed some evidence of carcinogenicity in laboratory animals. Other data indicate that Hydroquinone may have immunotoxic effects, especially on bone marrow. In addition, new data indicate that, although Hydroquinone has been shown to be a "slow" permeant through human skin using an aqueous vehicle, it is readily absorbed through the skin from an alcoholic vehicle.

Currently, some marketed hair dye formulations that use Hydroquinone also contain one or more alcohols. However, the Expert Panel noted that data supplied by industry indicate that during the hair dyeing procedure, Hydroquinone is a

"consumable." This means that the actual concentration of Hydroquinone decreases sharply as the color-forming reaction proceeds. Therefore, the amount of Hydroquinone that may be absorbed during the hair dyeing process is limited both by the decreasing concentration of available Hydroquinone and by the length of time the hair dye is applied before being rinsed off.

However, the actual exposure to and absorption of Hydroquinone as it is used in hair dyes would not be applicable to leave-on cosmetics. For leave-on products, absorption could occur over a period of time, even with products that do not contain alcohol. Also, in the case of lipstick formulations, Hydroquinone would be ingested and absorbed more readily.

Therefore, the Expert Panel determined that the CIR Expert Panel's conclusion of safety for the use of Hydroquinone in cosmetic products should distinguish between the two different types of exposure. The Expert Panel confirmed its original conclusion of safety for rinse-off preparations, but added the qualification indicating that Hydroquinone was safe for use in *aqueous* rinse-off products at $\leq 1.0\%$. The Expert Panel noted that the regulated drug use of Hydroquinone was, at least in part, for a cosmetic purpose, i.e., to eliminate blemishes of the skin, and/or for other medical conditions, such as uneven skin pigmentation. The Expert Panel therefore decided to clarify its earlier conclusion of 1986 in which all then-reported leave-on cosmetic uses of Hydroquinone were assumed to be misclassified and considered drugs, by now stating that Hydroquinone should not be used in any leave-on type of nondrug cosmetic product. Manufacturers of leave-on drug/cosmetic products that contain Hydroquinone should seek guidance from the governmental agencies that regulate such products.

CONCLUSION

The CIR Expert Panel concludes that Hydroquinone is safe at concentrations of 1.0% and less for *aqueous* cosmetic formulations designed for discontinuous, brief use followed by rinsing from the skin and hair. Hydroquinone should not be used in leave-on nondrug cosmetic products.

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