Chronic and Initiation/Promotion Skin Bioassays of Petroleum Refinery Streams

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Nine refinery streams were tested in both chronic and initiation/promotion (I/P) skin bioassays. In the chronic bioassay, groups of 50 C3H/HeJ mice received twice weekly applications of 50 µl of test article for at least 2 years. In the initiation phase of the I/P bioassay, groups of CD-1 mice received an initiating dose of 50 µl of test article for 5 consecutive days, followed by promotion with 50 µl of phorbol-12-myristate-13-acetate (0.01% w/v in acetone) for 25 weeks. In the promotion phase of the I/P bioassay, CD-1 mice were initiated with 50 µl of 7,12-dimethylbenzanthracene (0.1% w/v in acetone) or acetone, followed by promotion with 50 µl of test article twice weekly for 25 weeks. The most volatile of the streams, sweetened naphtha, and the least volatile, vacuum residuum, were noncarcinogenic in both assays. Middle distillates, with a boiling range of 150–370°C, demonstrated carcinogenic activity in the chronic bioassay and acted as promoters but not initiators in the I/P bioassay. Untreated mineral oil streams displayed initiating activity and were carcinogenic in the chronic bioassay, presumably due to the presence of poly cyclic aromatic hydrocarbons of requisite size and structure. A highly solvent-refined mineral oil stream lacked initiating activity. These results indicate that the I/P bioassay, which takes 6 months to complete, may be a good qualitative predictor of the results of a chronic bioassay, at least for petroleum streams. Furthermore, the I/P bioassay can provide insight into possible mechanisms of tumor development. Key words: initiation-promotion, petroleum products, refinery streams, skin painting bioassay. Environ Health Perspect 102:82-87(1994)

Mouse skin painting has long been used to assess the dermal carcinogenic potential of natural and synthetic materials (1–4). Compounds derived from petroleum were among the early materials tested by mouse skin painting and were important in demonstrating the carcinogenic potential of compounds such as polycyclic aromatic hydrocarbons (PAHs) of specific structure and elucidating the structure–activity relations now recognized for these compounds.

Early investigators (2,5) painted the skin of mice for long periods of time until or after the appearance of tumors. Modern investigators using this technique apply materials for periods approximating the lifetime of the mouse, particularly since promulgation of the FDA and EPA Good Laboratory Practice Regulations (6,7). It was learned that some materials could cause tumors after a few (or single) applications but only after subsequent longer-term administration of substances that were not necessarily active in the absence of the initial material (8,9). The active materials applied first came to be known as initiators, and those applied subsequently for a longer term were termed promoters. In time, a more or less standardized assay was developed to shorten the time required to identify carcinogens and to discriminate between initiators and promoters (10). This assay, known as the initiation/promotion (I/P) bioassay entails the application of a known or suspected tumor initiator, followed by multiple weekly treatments of a known or suspected tumor promoter.

The I/P bioassay is designed to differentiate substances that produce tumors by genotoxic mechanisms, nongenotoxic mechanisms, or both. Initiation is generally agreed to occur when one or a few cells of a tissue have been exposed to a carcinogen at a level sufficient to cause a preancerous, heritable, somatic, genetic event. A number of characteristics define the initiated cell, including a permanent change in its DNA. Promotion is thought to involve a number of changes in an initiated cell, causing the initiated cell to grow faster than the surrounding normal cells and to develop into a visible neoplasm. Initiating and promoting agents alone do not cause neoplasms to appear, or only rarely so, but in succession elicit both benign and malignant neoplasms.

A number of researchers have investigated the carcinogenicity of various petroleum fractions (11–15). They have documented the initiating potential and carcinogenicity of streams containing PAHs of characteristic structure and molecular weight. They have also shown that some fractions, which do not contain significant concentrations of PAHs, are tumorigenic in long-term assays and are promoters but not initiators in the I/P bioassay.

The American Petroleum Institute (API) assembled a task force of petroleum company scientists and engineers to identify the various streams produced in petroleum refineries for which carcinogenicity data would be most useful. These were tested chronically, and a subset, reported in this paper, was also tested using an I/P protocol to accomplish the following objectives: 1) to investigate the carcinogenic potential of a number of petroleum streams of varying volatility and composition; 2) to identify the active streams as promoters or initiators as defined by the I/P test protocol; 3) to investigate the correlation between results obtained in chronic studies and in I/P studies to evaluate the I/P bioassay as a possible less expensive and shorter alternative to the lifetime bioassay.

Methods

Chronic Study

The test articles listed in Table 1 were obtained from the API repository (Experimental Pathology Laboratories, Herndon, Virginia). These materials were among those that had been selected by a task force of the API to represent petroleum streams produced during petroleum refining. These streams have been characterized for inclusion in the EPA listing of materials covered by Toxic Substances Control Act regulations and given Chemical Abstract Service (CAS) identification numbers. Table 1 is a listing of the streams, their CAS numbers, and boiling ranges. Toluene, ACS grade, and benz[e]pyrene (BaP) were purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin).

Vacuum residuum was applied as a 50% (w/v) solution in toluene, and catalytic cracked clarified oil was applied as a 1% (w/v) solution in toluene. All other test articles were applied neat. We used BaP, a well-known carcinogenic component of crude petroleum, as the positive control. BaP was applied as a 0.05% (w/v) solution in toluene.

Male C3H/HeJ mice are most frequently used in chronic dermal studies of...
petroleum-derived materials (11,13,14) and therefore were used in this study. Mice were acquired from Jackson Laboratories (Bar Harbor, Maine). Mice were 7–9 weeks of age at study initiation. We randomized mice upon receipt and double housed them in suspended stainless-steel cages for a quarantine period and then housed them singly before study initiation. Some groups were quarantined for 2 weeks and held for a week, others were quarantined for 1 week and held for 2. Purina certified rodent chow #5002 was provided ad libitum. Deionized tap water was provided ad libitum by bottle. Animal rooms were maintained at 24.5 ± 1.7°C (SD) and relative humidity at 40% ± 10% (SD). A 12-hr light/dark cycle was maintained.

We randomly divided 600 mice into groups of 50 mice each. Groups of 50 mice are routinely used in chronic skin painting bioassays. In addition to the test groups, one group received no treatment, and one was administered BaP (0.05% w/v in toluene) as the positive control. Because two samples were diluted with toluene, a separate toluene group was included as a vehicle control. We applied the test articles, BaP, and toluene as 50-µl doses twice weekly for a lifetime, or in the case of three samples, 24 months. These three samples showed significantly high tumor incidences and short latencies to median tumor, so the shorter exposure period did not affect the classification of their carcinogenic activity. All mice were sacrificed by ethyl ether inhalation and exsanguinated by puncturing the posterior vena cava.

We applied all materials to the shaved, intrascapular region of the back. Mice were clipped approximately every other week. Materials were applied with a mechanical pipet with disposable syringe and/or pipet tip and allowed to spread evenly without any intervention.

We performed physical examinations for masses weekly. All mice were subjected to a limited necropsy upon death or sacrifice. We removed application-site skin and fixed it in 10% neutral buffered formalin. Skin specimens were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Significance of differences between test and control tumor incidences was analyzed by chi square (16). Fisher’s exact test (one-tailed) was used when comparing low-tumor-incidence groups (five or fewer mice with tumors) to the appropriate control. Final effective number (FEN) is the denominator used in calculating tumor incidence. When time to median tumor was under 60 weeks, we used the number of animals alive at that time as the FEN. When time to median tumor was greater than 60 weeks, the number of animals alive at 60 weeks plus any mice dying with tumors before 60 weeks was used as the FEN. Latency was measured as the time in weeks from initiation of dosing to appearance of the first tumor.

Initiation/Promotion Study

The test articles listed in Table 1 were stored refrigerated in 25-ml glass bottles. At 2-week intervals the test articles were removed from the refrigerator for use. Other reagents were purchased commercially: 7,12-dimethylbenzanthracene (DMBA) from Eastman Kodak (Rochester, New York), acetone, ACS grade, fromEK Industries (Addison, Illinois), phorbol-12-myristate-13-acetate (PMA) from LC Services Corporation (Woburn, Massachusetts), and toluene, ACS grade, from Fischer Scientific (Itasca, Illinois).

Vacuum residuum was applied as a 50% (w/v) solution in toluene, and the catalytic cracked clarified oil was applied as a 1% (w/v) solution in toluene. All other test articles and toluene were applied neat. DMBA, the usual initiator in promotion investigations, was prepared once time only in acetone at a concentration of 0.1% (w/v). We prepared PMA in acetone every 2 weeks at a concentration of 0.01% (w/v) and stored it protected from light at approximately 0°C.

Male CD-1 mice were routinely used as test animals in I/P studies (12,15) and were used in this investigation to permit comparison with previous studies. Male CD-1 mice were acquired from Charles River Breeding Laboratories (Portage, Michigan). Mice were 4–5 weeks old upon receipt and 7–9 weeks old at study initiation. They were determined to be free of viral antibodies upon arrival and were quarantined for at least 2 weeks before study initiation. Animals were housed singly at the time of randomization for study initiation and remained housed singly throughout the study in suspended stainless-wire cages. Rodent chow 5001 (Ralston Purina Co., St. Louis, Missouri) and tap water were provided ad libitum by means of an automatic watering system. The animal room temperatures were maintained at 21.3°C ± 1.6 (SD), and relative humidity at 54.5% ± 13.3 (SD). Animal rooms were illuminated with fluorescent lights and maintained on a 12-hr light/dark cycle.

In the initiation phase, we randomly divided 360 mice into 12 groups of 30 each. This number of mice per group has been shown to provide satisfactory sensitivity and selectivity in I/P studies (12,15). In addition to the nine test articles, acetone was used as a negative control, and DMBA was the positive control. Because two samples were diluted in toluene, a separate toluene-initiated group was included as a vehicle control. The test articles, acetone, and toluene were applied as five consecutive daily applications of 50 µl. In the positive control group, 50 µl of DMBA (0.1% w/v in acetone) was applied once on the last day of the dosing week. After a 2-week rest period, 50 µl of PMA promoter (0.01% w/v in acetone) was applied to each animal twice weekly for 25 weeks. Physical examinations including observations of dermal masses were performed weekly on all surviving mice until study completion. We sacrificed all mice by intraperitoneal injection of nembutal the week of PMA treatment termination, subjected mice to gross necropsy, and took skin sections for histopathological examination.

In the promotion phase, we randomly divided 630 mice into 21 groups of 30 mice each. Test article groups were the same as for initiation except that neither acetone nor PMA was tested as promoters. A negative control was added (sham-handled), which received no treatments after initiation. All mice were initiated with 50 µl of DMBA (0.1% w/v in acetone). In addition, each mouse in an identical series of test article groups of 30 mice each, except the sham-handled group, was treated with a single 50-µl dose of acetone as a negative initiating agent. After a 2-week rest period, the mice in each DMBA or acetone-initiated group received 50 µl of the test articles or toluene twice a week for

| Table 1. Petroleum streams samples examined |
|--------------------------------------------|
| Petroleum stream                          | CAS no.   | Boiling range, °C |
|--------------------------------------------|-----------|-------------------|
| Solvent-refined heavy naphthenic distillate| 64741-96-4| 321–547           |
| Hydrodesulfurized kerosene                | 64742-81-0| 183–279           |
| Sweetened naphtha                         | 64741-87-3| 37–128            |
| Hydrodesulfurized middle distillate       | 64742-80-9| 172–344           |
| Vacuum residuum                           | 64741-56-6| 343               |
| Catalytic cracked clarified oil           | 64741-62-4| 350               |
| Straight-run middle distillate / light catalytic cracked distillate| 64741-44-2 / 64741-59-9| 181–327 / 240–372     |
| Light catalytic cracked distillate        | 64741-59-9| 240–372           |
| Light paraffinic distillate               | 64741-50-0| 304–428           |

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whether with the acetone-initiated groups and comparisons. The outcome measures were 1) time to appearance of first tumor, 2) number of animals with clinically observed tumors, and 3) number of animals with histologically confirmed tumors. Statistical significance of treatment effect in the initiation study was assessed by comparison with the acetone-initiated group. In the promotion study, each sample had its own DMBA- and acetone-initiated groups and comparisons were made between them. We used two different statistical methods in the analysis of these data. For the time to first tumor data, a product-limit survival analysis (17) was used to contrast the survival distributions for the desired pairwise group comparisons. To analyze the number of animals with tumors, a one-tailed Fisher’s exact test (18) was performed to determine whether the incidence of histologically confirmed tumors in the sample group was higher than its respective control. The type I error rate used for all statistical comparisons was 5%.

Results
Irritation and inflammation at the dosing site were common in the exposed groups, with the most severe signs noted in mice exposed to light catalytic, cracked distillate, hydrodesulfurized middle distillate, and the mixture of 50% straight run, middle distillate/50% light catalytic, cracked distillate. A number of skin tumor types were noted: benign neoplasms classified as fibromas, papillomas, keratoacanthomas, and hemangiomas, and malignant neoplasms including squamous cell carcinomas, fibrosarcomas, and malignant melanomas. The incidence of keratoacanthomas, hemangiomas, and malignant melanomas was extremely low. Only one mouse in each of two different groups developed hemangiomas and only one a keratoacanthoma. Two mice in one group had malignant melanomas. At the time of micropathological examination, most of the tumors were malignant. This is not inconsistent with expectations based on a two-stage model of carcinogenesis suggesting that initiated (first stage) cells have an increasing probability of undergoing a second stage (malignant) change as the mouse ages. The results are given in Table 2.

The samples in this study represent complex mixtures derived from distillation of petroleum and different refining treatments. The most volatile, sweetened naphtha, does not demonstrate significant tumorigenic potential. This is also true for vacuum residuum, a nonvolatile fraction containing high molecular weight compounds including PAHs. Since this stream was diluted 50% in toluene, statistical comparison was made with the toluene solvent control rather than acetone.

BaP, a strong dermal carcinogen, produced tumors in virtually all exposed animals in this study at a concentration of 0.05%. The only petroleum stream that approached it in activity was catalytic, cracked clarified oil, which achieved 100% incidence at a concentration of 1%. Solvent-refined heavy naphthenic distillate lacked carcinogenic activity in the chronic study.

Except for light paraffinic distillate, the rest of the samples can be characterized as middle distillates with boiling ranges in the region of 150°–370°C. These streams appear to be tumorigenic after chronic dermal application under the conditions of this experiment. The skins of mice chronically exposed to the middle distillate samples evidenced severe irritation with inflammation, some erosion, and scab formation.

The tumors seen in the I/P study were overwhelmingly benign squamous cell papillomas and keratoacanthomas. The only malignant tumors, squamous cell carcinomas, occurred in two mice of the group promoted with light catalytic cracked distillate. Table 3 details the tumor incidence and latency after initiation and promotion phases of this assay.

The only samples manifesting initiating activity, as shown in Table 3, were 1% catalytic cracked clarified oil and light paraffinic distillate. The catalytic cracked clarified oil was almost as potent as 0.1% DMBA. Catalytic cracked clarified oil does not appear to have promoting potential, although this lack of activity may be a result of the low concentration tested (1%). Light paraffinic distillate appears to have promoting as well as initiating potency.

The middle distillate samples (hydrodesulfurized kerosene, hydrodesulfurized middle distillate, light catalytic cracked distillate, and the 50/50 mixture of straight run middle distillate/light catalytic cracked distillate) acted as pure promoters in the I/P assay with no significant initiating potential. Lastly, a heterogeneous trio of samples, sweetened naphtha, vacuum residuum, and solvent-refined heavy naphthenic distillate, failed to demonstrate initiating or promoting activity.

In the initiating phase, there was some inverse correlation between tumor incidence and latency with exceptions such as vacuum residuum, which had a low incidence and low latency. This did not appear to be the case in the promoting phase, however, where the latencies were quite similar for all tested groups developing tumors.

As noted previously, the only tumors occurring in chronic and I/P studies at higher than incidental rates were squamous cell carcinomas, squamous cell papillomas, fibrosarcomas, and keratoacanthomas. Table 4 lists the incidence rates of these tumors in both studies. The bulk of the neoplasms in the chronic study were squamous cell carcinomas with a significant representation of the fibrosarcomas and a relatively small incidence of squamous cell papillomas. As noted previously, all the tumors occurring in test groups in the I/P removal of...
Table 3. Initiating and promoting potential of petroleum streams

| Treatment groups         | Initiating phase | Promoting phase |
|--------------------------|------------------|-----------------|
|                          | Incidence \(^a\) | Latency (weeks) | Incidence \(^a\) | Latency (weeks) |
| Sham-handled             | NT               | NT              | 0              | —               |
| Hydrodesulfurized kerosene | 3/30             | 25              | 22/30*         | 14**            |
| Sweetened naphtha        | 3/29             | 20              | 0              | —               |
| Hydrodesulfurized middle distillate | 6/30            | 18              | 16/30*         | 13**            |
| Vacuum residuum          | 7/30             | 10              | 0              | —               |
| Catalytic cracked clarified oil, 1% w/v | 26/30*          | 9**             | 5/29           | 8**             |
| Solvent-refined heavy naphthenic distillate | 4/30             | 22              | 0              | —               |
| Straight-run middle distillate | 5/29             | 13              | 18/30*         | 13**            |
| Light catalytic cracked distillate 50/50 | 9/30            | 15              | 28/30*(1)b    | 11**            |
| Light paraffinic distillate | 14/30*          | 12**            | 8/29*          | 12**            |
| Toluene                  | 8/29             | 13**            | 2/30           | 26              |
| Acetone                  | 3/30             | 16              | NT             | NT              |
| DMBA, 0.1% w/v           | 30/30 (1)b       | 9**             | NT             | NT              |

Abbreviations: NT, not tested; DMBA, 7,12-dimethylbenzanthracene.

\(^a\)Incidence = number of mice with tumors/number surviving 20 weeks of exposure.

\(^b\)Number of malignant tumors in parentheses.

\(^c\)Significantly higher than its acetone control (\(p < 0.05\)) using one-tailed Fisher's exact test.

\(^d\)Significantly different from acetone control (\(p < 0.05\)) using Kaplan-Meier test.

Study were benign squamous cell papillomas and keratoacanthomas, with the exception of two mice with squamous cell carcinomas in the groups exposed to light catalytic cracked distillate. Aside from the fibrosarcomas, these were tumors of epidermal origin that might be expected to arise from dermal treatment with a tumorigen.

Discussion

Three streams in this study did not show statistically significant carcinogenic activity chronically or in I/P tests: sweetened naphtha, solvent-refined heavy naphthenic distillate, and vacuum residuum. Sweetened naphtha, a volatile fraction boiling in the range of 37°–128°C, was made mostly of paraffinic hydrocarbons, some naphthenes, and a low concentration of aromatics. The low boiling range of this stream implies that it will have a short residence time on the skin after application. Moreover, over analysis of the stream revealed no known carcinogenic components. The lack of activity may be due to one or both of these factors. Vacuum residuum is the least volatile of the streams and does not begin to distill below 350°C. No analytical data were available on this stream, but it is expected to contain some high molecular weight PAHs. These compounds may not be carcinogenic, explaining the innocuous character of the stream. Alternately, any active materials in vacuum residuum, if they exist, may not be available to the skin cells at risk for carcinogenic transformation. Lewis (14) has reported similar results from the nonvolatile asphalt fraction of crude oil. Solvent-refined heavy naphthenic distillate is a mineral oil that has been extracted to remove any carcinogenic PAHs. Kane et al. (13), Doak et al. (19), and Halder et al. (20) have reported that intensive solvent refining is effective in eliminating the carcinogenic potency of mineral oils. IARC has affirmed that judgment with the statement, "There is no evidence that severely solvent-refined oils are carcinogenic to experimental animals" (21: 151).

Two streams, catalytic cracked clarified oil and light paraffinic distillate, exhibited carcinogenic activity in the chronic study and were classified as initiators in the I/P study. Catalytic cracked clarified oil is a residual fraction from distillation of the products of a catalytic cracking process. It is likely to contain active PAHs, some of which may be heterocyclic. The lack of activity of this stream as a promoter may be a result of its dilution (1%) rather than any intrinsic property of the stream itself. Light paraffinic distillate is a mineral oil of relatively low viscosity with a high enough boiling range that it may contain low concentrations of PAHs, which may be responsible for its activity. The initiating activity of this stream is weak compared to that of the DMBA-positive control and catalytic clarified oil, reflecting the weak carcinogenicity relative to these materials in the chronic study.

The middle distillate samples (hydrodesulfurized kerosene, hydrodesulfurized middle distillate, light catalytic cracked distillate, and the 50/50 mixture of straight run middle distillate and light catalytic cracked distillate) were all consistent in their activities. They all led to tumor formation on chronic application, with a rather narrow range of incidence rates, and they proved to be promoters but not initiators in the I/P bioassay. Other studies have documented similar activity in middle distillate fractions and streams (11, 12, 14, 22). The concentrations of active PAHs in these streams are normally too low to be responsible for middle distillate tumorigenic potency (11), and some studies (12, 23) have reported that they act as promoters but not initiators as defined in a standard I/P bioassay.

Of the tumor types listed in Table 4, the squamous cell papillomas and carcinomas and the keratoacanthomas are epidermal in origin, whereas the fibrosarcomas derive from mesodermal tissue. The squamous cell carcinomas found in the chronic study might reasonably be regarded as successors to the papillomas and keratoacanthomas noted in the shorter-term I/P study, representing the malignant stage after early benign epidermal tumors. The fibrosarcomas, however, cannot be so regarded, and their appearance in the chronic study is difficult to interpret. The mouse strains in the study were different, C3H in the chronic study and CD-1 in the I/P study, and the fibrosarcomas may represent a peculiar reaction of the C3H strain.

Table 4. Incidence (%) of tumor types in chronic and initiation/promotion (I/P) studies

| Stream (exposure time) | Chronic study | I/P study |
|------------------------|---------------|-----------|
|                        | SCC | SCP | FS | I | P | I | P |
| Hydrodesulfurized kerosene (L) | 36  | 2   | 10 | 10 | 63 | 0 | 20 |
| Sweetened naphtha (L)    | 2   | 4   | 0  | 10 | 0  | 0 | 0  |
| Hydrodesulfurized middle distillate (L) | 46  | 2   | 12 | 20 | 53 | 0 | 7  |
| Vacuum residuum (L)      | 6   | 0   | 2  | 20 | 4  | 3 | 0  |
| Catalytic cracked clarified oil, 1% (L) | 74  | 8   | 14 | 83 | 17 | 23 | 0  |
| Solvent refined heavy naphthenic distillate (L) | 8   | 0   | 0  | 13 | 0  | 0 | 0  |
| Straight-run middle distillate/light catalytic cracked distillate, 50/50 (L) | 22  | 8   | 6  | 14 | 60 | 7 | 10 |
| Light, catalytic cracked distillate (24) | 54  | 14  | 24 | 27 | 90 | 10 | 33 |
| Light paraffinic distillate (24) | 54  | 12  | 16 | 40 | 28 | 17 | 0  |
| Toluene (24)             | 0   | 0   | 0  | 24 | 7  | 3 | 0  |
| Toluene (L)              | 6   | 0   | 2  | 24 | 7  | 3 | 0  |
| BaP (24)                 | 32  | 4   | 62 | 63 | 10 | 50 | 6  |
| BaP (L)                  | 68  | 0   | 44 | 0  | 0  | 0 | 0  |

Abbreviations: SCC, squamous cell carcinoma; SCP, squamous cell papilloma; FS, fibrosarcoma; KA, keratoacanthoma; BaP, benzo[a]pyrene.

\(^L\) Life time; 24, 24 months.
after dermal exposure to carcinogens. However, Biles et al. (11) used C3H mice in
their study of chronic dermal application of middle distillate samples and iden-
tified only one fibrosarcoma in 490 mice in the 10 groups tested. It appears that
the C3H strain is not consistently susceptible to the development of fibrosarcomas.
In any event, the appearance of fibrosarcomas in the chronic study does not affect
the good correlation of the I/P study with the chronic investigation as far as tumorigenic
activity is concerned.

Table 5 summarizes the activity of the various petroleum streams tested in this
investigation in both chronic and I/P stud-
ies. The results of the chronic bioassay in
this investigation are consistent with previ-
ous studies (11,13,14), which have shown
the following: 1) the most volatile streams
are inactive, 2) the least volatile residues
(vacuum residuum) are also inactive, 3)
untreated distillates (light paraffinic distillate
in this study) are active but may be
made inactive by suitable treatment to
remove PAHs (solvent-refined heavy naph-
thenic distillate in this study), 4) middle
distillates show carcinogenic activity in
chronic dermal studies.

The I/P investigations carried out in
this study demonstrate convincingly that
the middle distillates with carcinogenic
activity in the chronic study act as prom-
oters and not as initiators. This too supports
results of previous investigations (12,15).
The activity of the two streams showing
initiation potential, 1% catalytic cracked
clarified oil, and light paraffinic distillate,
may result from the presence of active
PAHs and/or polyaromatic heterocycles in
the case of catalytic cracked clarified oil.

The I/P portion of the investigation
demonstrated perfect correlation with the
chronic phase in that all streams negative in
the chronic bioassay were inactive as ini-
tiators and promoters, and all streams posi-
tive in the chronic bioassay were active in
either the initiation phase or promotion
phase or both. This indicates, at least for
petroleum streams, that the I/P bioassay
may be a cost-effective substitute for the
chronic test. Results would be available in
6 months rather than 2 years, providing
earlier information at a lower cost. It would
also furnish additional information with
regard to the nature of the carcinogenic
activity, which might be useful in assessing
hazard.

The significance of the distinction
between initiation and promotion in regard
to risk assessment of petroleum streams
remains to be clarified, and an API consor-
tium is currently sponsoring research in the
field. Over a number of years, hypotheses
have been put forth regarding the develop-
ment of at least some cancers as a two-stage
process (24–27). These authors and their
co-workers have demonstrated that the
time course of tumor incidence can be suc-
cessfully modeled using this approach for
naturally occurring tumors in humans (27)
or for chemically induced cancers in experi-
mental animals (24,28). One element of
great significance in the model is cellular
proliferation, with critical importance asso-
ciated with the particular cell stage affected
and the schedule of proliferation. If initia-
tion in the I/P bioassay is considered to
correspond to initiation in the two-stage
model and promotion to providing a prolif-
erative advantage to the initiated cells, skin
carcinogenic activity by promoters can be
examined using this model.

McKee et al. (15) have documented that
skin irritation and hyperplasia are caused by
repeated application of middle distillates to
mouse skin, and Skisak (23) has shown that
elimination of these effects can reduce or
eliminate tumor development after middle
distillate exposure. If compensatory hyper-
plasia provides a proliferative advantage to
initiated cells, the dermal carcinogenic activ-
ity of middle distillates can be visualized as
promotion of cells initiated by exposure to
background environmental mutagens. One
consequence of application of the two-stage
model is that the results of a chronic bioas-
say of a promoter cannot be applied un-
critically to risk assessment (25). Because
cellular proliferation is a function of the der-
al application schedule, which is different
in the experimental situation than in human
experience, direct extrapolation will proba-
bly lead to erroneous conclusions.

Table 5. Comparative chronic and initiation/promotion activity of petroleum streams

| Stream                        | Chronic | Initiation | Promotion |
|-------------------------------|---------|------------|-----------|
| Hydrodesulfurized kerosene    | –       | –          | +         |
| Sweetened naphtha             | –       | –          | –         |
| Hydrodesulfurized middle distillate | –     | –          | +         |
| Vacuum residuum               | –       | –          | –         |
| Catalytic cracked clarified oil, 1% | +     | +          | –         |
| Solvent-refined heavy naphthenic distillate | +   | +          | –         |
| Straight run middle distillate | –       | –          | –         |
| light catalytic cracked distillate, 50/50 | +   | –          | –         |
| Light catalytic cracked distillate | +     | +          | +         |
| Light paraffinic distillate   | +       | +          | +         |

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