Recent Findings on the Carcinogenicity of Chlorinated Olefins

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Data are presented on factors affecting the carcinogenic effects of chlorinated olefins, such as molecular structure, concentration, length of treatment, route of administration and animal species, strain, sex, and age. The observations are based upon carcinogenicity experimental bioassays of vinyl chloride and vinylidene chloride.

Early results, which appear to show that some of these factors (particularly species, strain, and sex) act by affecting the metabolism of the tested compounds, are presented, and the need for metabolic characterization of experimental animals in chemical carcinogenesis is stressed.

A project of integrated research on the carcinogenicity of different halogenated and related compounds has been going on at the Bologna Institute of Oncology and Tumour Center since 1971, and additional programs in this area are under way or under study.

Past and ongoing experiments deal with vinyl chloride (VC), vinylidene chloride (VDC), styrene, acrylonitrile, dichloroethane, chlorofluorocarbons, trichloroethylene, carbon tetrachloride, and vinylidene fluoride.

The experiments were performed by the same group in a very standard way, thus providing homogeneous and comparable information.

One is now becoming increasingly more aware that a great deal of this information may help not only to learn the effects of the particular compounds under study and their mechanism of action, but to better understand the basic factors involved in carcinogenesis in general.

Factors Affecting Neoplastic Response

The present report deals with the results obtained in our studies on the carcinogenicity of VC and VDC, pointing out the major factors affecting the neoplastic response.

The factors considered are: molecular structure, concentration, length of treatment, route of administration, animal species, strain, sex, age, and their relevance will be illustrated with VC and VDC.

Influence of the Molecular Structure

By comparing the carcinogenic activity of VC with that of VDC it can immediately be seen that molecules of very similar structure, such as vinyl chloride and vinylidene chloride, may have very different biological effects (Table 1).

While VC has been shown to affect many organs of different animal species, i.e., to be a clear multipotential carcinogen, VDC, to the present, has produced oncogenic effects definitively only in one organ (kidney) of a single species (mouse) (1-4).

Vinyl Chloride:

\[ CH_2 \equiv CHCl \]

Vinylidene Chloride:

\[ CH_2 \equiv CCl_2 \]

Influence of Concentration

The studies on VC have already confirmed the dose-response relationship effect (1-3). Furthermore, they show that different concentrations, though within a narrow range of dose, may greatly affect the relative proportion of different types of tumors caused by VC (Table 2).
Table 1. Effects of chemical structure: comparative oncogenic effects of VC and VDC on rodents.

| Compound | Species       | Angiosarcomas of liver | Angiosarcomas and angiomas of other sites | Nephroblastos | Adenocarcinomas of kidney | Sebaceous carcinomas | Other cutaneous skin tumors | Tumors of lung | Tumors of brain | Mammary carcinomas | Forestomach papillomas and acantomas | Lymphomas and leukemias |
|----------|---------------|------------------------|------------------------------------------|--------------|--------------------------|---------------------|----------------------------|-----------------|-----------------|---------------------|--------------------------------------|------------------------|
| VC       | Rat           | +                      | +                                        | +            | +                        | +                   | +                         | +               | +               | +                   | +                                     | +                      |
|          | Mouse         | +                      | +                                        | +            | +                        | +                   | +                         | +               | +               | +                   | +                                     | +                      |
|          | Hamster (Golden) | +              | (+)                                      |              |                          |                     |                           |                 |                 |                     |                                       |                        |
| VDC      | Rat           |                        |                                          |              |                          |                     |                           |                 |                 |                     |                                       |                        |
|          | Mouse         |                        |                                          |              |                          |                     |                           |                 |                 |                     |                                       |                        |
|          | Hamster (Chinese) |                        |                                          |              |                          |                     |                           |                 |                 |                     |                                       |                        |

"Including Zymbal gland carcinomas.

Table 2. Effects of concentration."

| Groups | Concentration of VC, ppm | No. of animals with tumors | Side of animal | | No. of animals with tumors | |
|--------|--------------------------|-----------------------------|----------------|--------------------------|------------------|
|        |                          |                             |                |                         |                  |
| I      | 200                      | 120                         | Liver angio     | 12                       | 3                 |
| II     | 150                      | 120                         | Angio           | 5                        | 7                 |
| III    | 100                      | 120                         |                 | 1                        | 10                |
| IV     | None                     | 120                         |                | 0                        | 0                 |

"Exposure by inhalation to VC in air, at 200, 150, 100 ppm, 4 hr/day, 5 days/week, for 52 weeks. (Results after 143 weeks = end of the experiments.)

Influence of the Route of Administration

The route of administration of VC may significantly vary the type of neoplastic response (Table 4), probably by affecting the distribution of the compound and its metabolites in the body.

Influence of Animal Species

The animal species is a very important factor in carcinogenesis. Studies on VC carcinogenesis have shown that the range of VC-dependent tumors greatly varies from species to species, though some, such as liver angiosarcomas, are observed in all species tested (1-3).

Recent result with VDC (4) have pointed out that...
the only type of tumor known at present as definitively VDC-dependent, the renal adenocarcinoma, is observed in mice but not in the other two tested species, i.e., rats and Chinese hamsters, though rats have been treated with higher doses (Table 5).

**Influence of Strain**

Differences have been observed among strain in VC carcinogenesis. As an example, the onset of Zymbal gland carcinomas, following the treatment with this monomer, varies greatly in Sprague-Dawley and Wistar rats, though the spontaneous onset is exceptionally rare even in the most sensitive strain (Table 6).

**Influence of Sex**

If VDC had been tested only on female Swiss mice, probably the knowledge of its capacity to produce kidney adenocarcinomas would not have come to light (Table 7).

**Influence of Age**

Hepatocarcinogenesis by VC is a striking example of the influence of age in neoplastic response (Table 8).

### Table 5. Effects of species.

| Groups | Concentration of VDC, ppm | Species and strain | Animals with kidney adenocarcinomas |
|--------|--------------------------|--------------------|-----------------------------------|
| I      | 200-150                  | Rats Sprague-Dawley | 120 0 —                            |
| II     | 100                      | Rats Sprague-Dawley | 60 0 —                             |
| III    | 50                       | Rats Sprague-Dawley | 60 0 —                             |
| IV     | 25                       | Rats Sprague-Dawley | 60 0 —                             |
| V      | None                     | Rats Sprague-Dawley | 200 0 —                            |
| VI     | 25                       | Mice Swiss          | 300 25 8.3                         |
| VII    | None                     | Mice Swiss          | 200 0 —                            |

*aExposure by inhalation to VDC in air at different concentrations, 4 hr/day, 4-5 days/week, for 52 weeks. (Results after 98 weeks = ongoing experiments.)*

### Table 6. Effects of strain.

| Groups | Concentration of VC, ppm | Animals (male rats) | Animals with Zymbal gland carcinomas |
|--------|--------------------------|--------------------|-------------------------------------|
| I      | 10,000                   | Sprague-Dawley     | 30 SD 3                             |
| II     | 10,000                   | Wistar             | 30 3 1                             |
| III    | 6,000                    | Sprague-Dawley     | 30 3 0                             |
| IV     | 6,000                    | Wistar             | 30 1 0                             |
| V      | 2,500                    | Sprague-Dawley     | 30 3 0                             |
| VI     | 2,500                    | Wistar             | 30 3 0                             |
| VII    | 500                      | Sprague-Dawley     | 30 3 0                             |
| VIII   | 500                      | Wistar             | 30 3 0                             |
| IX     | None                     | Sprague-Dawley     | 30 0 0                             |
| X      | None                     | Wistar             | 30 0 0                             |
| Total  |                          |                    | 17 1                                |

*aExposure by inhalation to VC in air at different concentrations, 4 hr/day, 4-5 days/week, for 52 weeks. (Results after 143 weeks = end of the experiments.)*
Explanation of Role of Factors

How the experimental and biological factors which have been considered and other possible ones determine the neoplastic response, is a matter of hypothesis and inference, more or less based upon experimental evidence.

To explain the influence of some biological parameters (such as species and strain), one has first to consider the genetic basis of responsiveness of different tissues and organs in various types of animals, often expressing itself in the onset of spontaneous neoplasia in the same tissue and organ. However it becomes more and more evident that experimental and biological factors affecting the neoplastic response in chemical carcinogenesis as well as other possible toxic effects may act by determining the metabolic pathway of the tested compounds.

Recent research has shown that VC and VDC do not act per se, but through products of metabolic transformation, probably epoxy-derivatives.

Experiments performed in our laboratories appear to indicate that species, strain and sex greatly affect the production of active metabolites, which in turn are responsible for toxic effects of VDC (Table 9 (5)).

The major regressive and necrotic changes produced by intoxication are found in liver and kidneys.

In the case of Sprague-Dawley rats and Swiss mice there is a clear cut parallelism between toxic and carcinogenic effects of VDC (in relation to species and sex).

As far as Balb/c, C3H, and C57BL mice are concerned, we are now undertaking long-term studies to assess if there is the same parallelism (in relation to strain).

Table 7. Effects of sex. a

| Groups | VDC treatment ppm | Animals (Swiss mice) | Animals with kidney adenocarcinomas |
|--------|------------------|----------------------|-----------------------------------|
|        |                  | Sex No. | No. | % |
| I      | 25               | male | 150 | 24 | 16 |
| II     | 25               | female | 150 | 1 | 0.7 |
| III    | None             | male | 190 | 0 | — |
| IV     | None             | female | 190 | 0 | — |

*Exposure by inhalation to VDC in air at 25 ppm, 4 hr/day, 4-5 days/week, for 52 weeks. (Results after 98 weeks = ongoing experiments.)

Table 8. Effects of age. a

| Groups | VC treatment, ppm | Age | No. of animals | No. of liver tumors |
|--------|------------------|-----|----------------|---------------------|
|        |                  |     | Total Surivors | Angiosarcomas Hepatomas |
| I      | 10,000           | 13 weeks | 120 | 16 |
| II     | 6,000            | 13 weeks | 120 | 15 |
| III    | None             | 13 weeks | 249 | 55 |
| IV     | 10,000           | 1 day | 46 | 8 |
| V      | 6,000            | 1 day | 43 | 5 |

*Incidence of hepatic tumors (angiosarcomas and hepatomas) among Sprague-Dawley rats, exposed to VC in air at 10,000 and 6,000 ppm, 4 hr/day, 5 days/week, for 5 weeks, at ages 13 weeks or 1 day. (Results after 135 weeks = end of the experiments.)

Table 9. Comparative acute toxic effects of vinylidene chloride (VDC), at 200 ppm in air, 4 hr/day for 2 days.

| Species | Strain | Sex | No. | 1 2 3 4 5 6 7 8 9 | Weight a | Performance status b |
|---------|--------|-----|-----|-------------------|----------|----------------------|
| Rats    | Sprague-Dawley | M | 60 | 60 60 60 60 60 59 | (+) | (+) |
|         |         | F | 60 | 60 60 60 60 60 60 | + | — |
| Mice    | Swiss  | M | 60 | 31 26 24 20 16 | ++ | ++ |
|         |        | F | 60 | 60 60 60 60 | - | — |
|         | Balb/c | M | 30 | 25 12 6 6 6 | - | ++ |
|         |        | F | 30 | 30 30 30 30 30 | - | — |
|         | C3H    | M | 30 | 30 25 17 17 15 | - | + |
|         |        | F | 30 | 22 19 19 19 | + | — |
|         | C57BL | M | 30 | 28 25 23 23 23 | — | +(+) |
|         |        | F | 30 | 30 30 30 30 | — | — |

*Code: —, decrease; =, no change; +, enhancement (on 5th day).

*Code: —, no effect; +, slight effect; ++, moderate effect; ++++, marked effect; +++++, profound effect. Recovery has been observed on nearly all the survivors on the 5th day.
Should it be confirmed, we do believe that there will be new routes for establishing priorities for long-term carcinogenicity bioassays, for choosing the best experimental animal models and for the understanding of mechanism of action of many organic carcinogens.

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