Carcinogenicity Bioassays of Vinyl Chloride Monomer: A Model of Risk Assessment on an Experimental Basis

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Data are presented regarding the final results of the Bentivoglio (Bologna) project on long-term carcinogenicity bioassays of vinyl chloride (VC).

The experimental project studied the effects of the monomer, administered by different routes, concentrations and schedules of treatment, to animals (near 7000) of different species, strains, sex and age. To our knowledge this is the largest experimental carcinogenicity study performed on a single compound by a single institution.

The results indicate that VC is a multipotential carcinogen, affecting a variety of organs and tissues. In the experimental conditions studied, the neoplastic effects of the monomer were also detected at low doses. The experimental and biological factors greatly affect the neoplastic response to VC. Long-term carcinogenicity bioassays are, at present, a unique tool for the identification and quantification of environmental and occupational risks. Precise and highly standardized experimental procedures are needed to obtain data for risk assessment.

Introduction

The present report deals with the presentation of the final results of our project on the long-term carcinogenicity bioassays of vinyl chloride (VC) (BT project).

To our knowledge this project is the most extensive experimental carcinogenesis study ever performed on one industrial compound by a single institution.

Planning, Materials, Methods and Performance of the Experiment

Planning

The experiments of the project were planned (a) to test the carcinogenicity of the compound; (b) to obtain information on the site and type of tumors; (c) to evaluate the possible effects of the routes of administration, with particular regard to the ones reproducing potential human exposure; (d) to assess, in quantitative terms, the level of risk. The planning of the experiments was aimed at achieving these goals.

The compound was tested on animals of different species, strain, sex and age (Table 1), since it is known that these factors may modify the neoplastic response qualitatively and quantitatively. The choice of the animals was made with the intention of having an integrated system of complementary biological models which could express a range, as wide as possible, of neoplastic responses.

VC was administered by different routes: intraperitoneal (IP) injection, subcutaneous (SC) injection, inhalation and ingestion (by stomach tube), the latter two being the major routes of potential human exposure.

The monomer was administered at different concentrations: 14 by inhalation levels and 6 ingestion levels for various periods of time, by continuous or intermittent treatment (Table 2).
The plan of the project is presented in Tables 3-9.

Material

VC was supplied from the same source in all cases, and it contained very low amounts of impurities (Table 10). The oil employed as a vehicle in the ingestion and injection experiments was pure virgin olive oil from Tuscany.

The animals (except for the golden hamsters) were breeds which have been routinely employed in our laboratory for many years. It should be pointed out that, whatever their use, all the animals of our colony undergo periodic examination and complete autopsy, giving us extensive information concerning their pathology.

The chambers for inhalation exposure were built basically of stainless steel and glass.

For the ingestion treatment glass syringes and stainless steel needles with round tips were used.

To control the level of exposure in the inhalation experiments, an automatic gas chromatography system was used.

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Table 1. BT project on VC: animals used.

| Species | Strain | Sex | Age            |
|---------|--------|-----|----------------|
| Rat     | Sprague-Dawley | M, F  | Adult (10-21 wk) |
|         |        |     | Newborn (1 day) |
| Rat     | Wistar | M   | Adult          |
| Mouse   | Swiss  | M, F | Adult          |
| Hamster | Golden | M   | Adult          |
|         |        |     | Embryo (12 days pregnancy) |

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Table 2. BT project on VC: routes, concentrations and schedules.

| Route         | Concentration | Schedule               |
|---------------|---------------|------------------------|
| Inhalation    | 30,000, 10,000, 6000, 2500, 500, 250, 200, 150, 100, 50, 25, 10, 5, 1 ppm | 4 hr/day, 5 days/wk, 52 wk |
|               | 10,000, 6000, 2500, 500, 250, 50 ppm | 4 hr/day, 5 days/wk, 17 wk |
|               | 10,000, 6000 ppm | 4 hr/day, 5 days/wk, 5 wk |
|               | 10,000, 6000 ppm | 4 hr/day, 1 day/wk, 25 wk |
|               | 10,000, 6000 ppm | 1 hr/day, 4 days/wk, 25 wk |
|               | 10,000, 6000 ppm | 4 hr/day, 7 days |
| Ingestion     | 50, 16.65, 3.33, 1.0, 0.3, 0.03, mg/kg body weight | 5 times/wk, 52 wk |
| IP injection  | 4.25 mg       | 4, 3, or 2 times at 2 month intervals |
| SC injection  | 4.25 mg       | Once only              |
|               |               |                        |

Table 3. Plan of long-term experiments on the effects of exposure by inhalation for 1 year to different doses of VC on adult Sprague-Dawley rats (basic experiments).

| Expt. no. | Route | VC dose | Duration | Treatment | Animals | Species | Strain | Age, weeks | No. ♀ | No. ♂ | Total | No. per group |
|-----------|-------|---------|----------|-----------|---------|---------|--------|------------|-------|-------|-------|---------------|
| BT1       | Inhalation | 10,000, 6000, 2500, 500 ppm | 4 hr/day, 5 days/wk, 52 wk | Rat Sprague-Dawley | 13 | 240 | 240 | 480 | 60 |
| BT2       | Inhalation | 200, 150, 100 ppm | 4 hr/day, 5 days/wk, 52 wk | Rat Sprague-Dawley | 13 | 280 | 265 | 545 | 120-185 |
| BT6       | Inhalation | 30,000 ppm | 4 hr/day, 5 days/wk, 52 wk | Rat Sprague-Dawley | 17 | 30 | 30 | 60 | 60 |
| BT9       | Inhalation | 50 ppm | 4 hr/day, 5 days/wk, 52 wk | Rat Sprague-Dawley | 11 | 200 | 200 | 400 | 100 (c) |
| BT15      | Inhalation | 25, 10, 5, 1 ppm | 4 hr/day, 5 days/wk, 52 wk | Rat Sprague-Dawley | 13 | 300 | 300 | 600 | 120 |

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Table 4. Plan of long-term experiments on the effects of length of VC exposure on VC carcinogenicity.

| Expt. no. | Route  | VC dose                  | Duration                                      | Species  | Strain         | Age, weeks | No. ♂ | No. ♀ | Total | No. per group |
|-----------|--------|--------------------------|-----------------------------------------------|----------|----------------|------------|-------|-------|-------|---------------|
| BT3       | Inhalation | 10,000, 6000, 2500, 500, 250, 50 ppm | 4 hr/day, 5 days/wk, 17 wk | Rat   | Sprague-Dawley | 12         | 262   | 288   | 550   | 60-190        |
| BT10      | Inhalation | 10,000, 6000 ppm Untreated controls | 4 hr/day, 5 days/ wk, 5 wk; 4 hr/ day, 1 day/wk, 25 wk; 1 hr/day, 4 days/wk, 25 wk | Rat  | Sprague-Dawley | 11         | 420   | 420   | 840   | 120           |

Table 5. Plan of long-term experiments on the effects of age on vinyl chloride carcinogenicity.

| Expt. no. | Route  | VC dose                  | Duration                                      | Species  | Strain         | Age, weeks | No. ♂ | No. ♀ | Total | No. per group |
|-----------|--------|--------------------------|-----------------------------------------------|----------|----------------|------------|-------|-------|-------|---------------|
| BT5       | Transplantal | 10,000, 6000 ppm | 4 hr/day, 7 days (from 12th to 18th day of pregnancy) | Rat   | Sprague-Dawley | 19         | 110   | 36    | 146   | 30-54         |
| BT14      | Inhalation | 10,000, 6000 ppm | 4 hr/day, 5 days/wk, 5 wk                     | Rat   | Sprague-Dawley | 1          | 45    | 44    | 89    | 43-46         |

Table 6. Plan of long-term experiments on the effects of strain on vinyl chloride carcinogenicity.

| Expt. no. | Route  | VC dose                  | Duration                                      | Species  | Strain         | Age, weeks | No. ♂ | No. ♀ | Total | No. per group |
|-----------|--------|--------------------------|-----------------------------------------------|----------|----------------|------------|-------|-------|-------|---------------|
| BT7       | Inhalation | 10,000, 6000, 2500, 500, 250, 50 ppm | 4 hr/day, 5 days/wk, 52 wk | Rat   | Wistar         | 11         | 0     | 220   | 220   | 30-40         |
| BT17      | Inhalation | 1 ppm Untreated controls | 4 hr/day, 5 days/wk, 52 wk                    | Rat   | Wistar         | 13         | 0     | 250   | 250   | 120-130        |

Table 7. Plan of long-term experiments on the effects of species on vinyl chloride carcinogenicity.

| Expt. no. | Route  | VC dose                  | Duration                                      | Species  | Strain         | Age, weeks | No. ♂ | No. ♀ | Total | No. per group |
|-----------|--------|--------------------------|-----------------------------------------------|----------|----------------|------------|-------|-------|-------|---------------|
| BT4       | Inhalation | 10,000, 6000, 2500, 250, 50 ppm Untreated controls | 4 hr/day, 5 days/wk, 30 wk                    | Mouse   | Swiss          | 11         | 500   | 250   | 510   | 60-150        |
| BT8       | Inhalation | 10,000, 6000, 2500, 500, 250, 50 ppm Untreated controls | 4 hr/day, 5 days/wk, 30 wk                    | Hamster | Golden         | 11         | 10    | 268   | 268   | 30-62         |
- Table 8. Plan of long-term ingestion experiments on vinyl chloride carcinogenicity.

| Expt. no. | Route     | VC dose                  | Duration       | Animals         |
|-----------|-----------|--------------------------|----------------|----------------|
| BT11      | Ingestion | 50, 16.65, 3.33 mg/kg body weight in olive oil Controls, olive oil | 5 times/wk, 52 wk | Rat Sprague-Dawley |
| BT27      | Ingestion | 1, 0.3, 0.03 mg/kg body weight in olive oil Controls, olive oil | 5 times/wk, 52 wk 59 wk | Rat Sprague-Dawley |

*For 10 animals of each of the three exposed and control groups the treatment was planned to last 104 weeks, but it had to be stopped because of animal intolerance.

- Table 9. Plan of long-term injection experiments on vinyl chloride carcinogenicity.

| Expt. no. | Route     | VC dose                  | Duration       | Animals         |
|-----------|-----------|--------------------------|----------------|----------------|
| BT12      | IP injection | 4.25 mg in 1.0 cc olive oil Controls, 1.0 cc olive oil | 4, 3, 2, or 1 times; two month intervals 1 injection | Rat Sprague-Dawley |
| BT13      | SC injection | 4.25 mg in 1.0 cc olive oil Controls, 1.0 cc olive oil |  | Rat Sprague-Dawley |

- Table 10. Maximum level of impurities in the VC used.

| Impurity        | Concen, ppm |
|-----------------|-------------|
| H₂O             | 10          |
| Acetic aldehyde | 5           |
| Acetylene       | 2           |
| Allene          | 5           |
| Butane          | 8           |
| 1,3-Butadiene   | 10          |
| Chlorophene     | 10          |
| Diacetylene     | 4           |
| Vinyl acetylene | 10          |
| Propine         | 3           |
| Methyl chloride | 100         |

- Modalities of Treatment. Treatment was always performed by the same person. This is particularly important for gavage, since the animals become accustomed to the same operator.

- Control of the Animals. The conditions of the animals were checked three times daily. Every two weeks the animals were examined to detect any gross changes.

- Weight of the Animals. The animals were weighed every two weeks during treatment and every eight weeks after the end of treatment.

- Duration of the Experiments. In the VC project, as in any other long-term bioassays performed in our laboratory, the animals were kept alive until spontaneous death.

- Autopsy. Full autopsy was performed on each animal. All parts of the body were explored, including the central nervous system. Specimens for histology included the brain, Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, liver, kidneys, adrenals, spleen, pancreas, stomach, intestine, bladder, uterus, gonads and any other organ with pathological lesions.

- Histology. Specimens were trimmed in the standard way. Sections were routinely stained with Haematoxylin-Eosin and, when necessary, with special techniques.

- Histopathological Examination. All sides were screened by a junior pathologist and then reviewed.

Methods and Procedures

For the experiment on VC, as well as for any other long-term experimental bioassays performed in our laboratory, the procedure has been always the same highly standardized and controlled one. In particular, the following points in our laboratory standard procedures, should be emphasized.

- Compounds. All shipments of VC used were examined in order to determine whether they meet the required standards.

- Concentrations. The concentrations, particularly when VC was given by inhalation, were controlled by continuous gas chromatographic monitoring.

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by a senior pathologist. The same classification of the lesions were used by all pathologists. 

Classification of Data. All the anatomical sites and the gross and microscopic observations were classified and coded following our laboratory codes (Tables 11-13).

Table 11. Codes of organs considered (sequence).

| Code | Organ                              |
|------|------------------------------------|
| 1    | Skin (epidermis and dermis)        |
| 2    | Epidermal appendages               |
| 3    | Zymbal glands                      |
| 4    | Subcutaneous tissues               |
| 5    | Mammary glands                     |
| 6    | Parotid glands                     |
| 7    | Submaxillary glands                |
| 8    | Nasal and paranasal cavities       |
| 9    | Oral cavity                        |
| 10   | Tongue                             |
| 11   | Lung                               |
| 12   | Pleura and pleural cavity          |
| 13   | Esophagus                          |
| 14   | Foregut stomach                    |
| 15   | Glandular stomach                  |
| 16   | Intestine                          |
| 17   | Liver                              |
| 18   | Pancreas                           |
| 19   | Peritoneum and peritoneal cavity   |
| 20   | Kidneys                            |
| 21   | Pelves                             |
| 22   | Ureters                            |
| 23   | Bladder                            |
| 24   | Ovaries                            |
| 25   | Uterus                             |
| 26   | Seminal vesicles                   |
| 27   | Prostate                           |
| 28   | Testicles                          |
| 29   | Epididymis                         |
| 30   | Hypophysis                         |
| 31   | Thyroid                            |
| 32   | Adrenals                           |
| 33   | Cerebrum                           |
| 34   | Cerebellum                         |
| 35   | Spinal marrow                      |
| 36   | Peripheral nervous system: ganglia |
| 37   | Peripheral nervous system: nerves  |
| 38   | Eyes                               |
| 39   | Harderian glands                   |
| 40   | Skeletal muscles (diaphragm not included) |
| 41   | Diaphragm                          |
| 42   | Bones                              |
| 43   | Articulations                      |
| 44   | Heart                              |
| 45   | Pericardium and pericardial cavity |
| 46   | Large vessels                      |
| 47   | Thymus                             |
| 48   | Spleen                             |
| 49   | Axillary and inguinal lymph nodes  |
| 50   | Head-neck lymph nodes              |
| 51   | Interthoracic and parathymic lymph nodes |
| 52   | Intrabdominal lymph nodes          |
| 53   | Lymph nodes of other sites         |
| 54   | Bone marrow                        |
| 55   | Soft tissues of support            |
| 56   | Interscapular fat pad              |
| 57   | Trachea                            |
| 58   | Ear                                |
| 59   | Female external sex organs         |
| 60   | Male external sex organs           |
| 61   | Odontogenic apparatus              |
| 62   | Gall bladder                       |

Table 12. Codes of macroscopic changes.

| Code | Change                              |
|------|-------------------------------------|
| 1    | No change                           |
| 2    | Alopecia                            |
| 3    | Keratosis                           |
| 4    | Degenerative pathosis               |
| 5    | Ucleation                           |
| 6    | Hyperemia, edema and hemorrhage     |
| 7    | Phlogosis (including of abscess)    |
| 8    | Pulmonary hepatization              |
| 9    | Pulmonary emphysema                 |
| 10   | Irregular surface                   |
| 11   | Granulations and plaques            |
| 12   | Simple thickening                   |
| 13   | Thickening of capsule               |
| 14   | Fibrosis                            |
| 15   | In toto reduction                   |
| 16   | Atrophy                             |
| 17   | In toto enlargement                 |
| 18   | Augmentation in consistency         |
| 19   | Dilatation of organ with cavity     |
| 20   | Protrusion of eyeball               |
| 21   | Simple cyst                         |
| 22   | Hemorrhagic cyst                    |
| 23   | Multiple simple cyst                |
| 24   | Multiple hemorrhagic cyst           |
| 25   | Polypoid formation                  |
| 26   | Papillomatous formation and horn    |
| 27   | Solid nodule                        |
| 28   | Hemorrhagic nodule                  |
| 29   | Cystic mass                         |
| 30   | Solid mass                          |
| 31   | Solid necrotic mass                 |
| 32   | Hemorrhagic mass                    |
| 33   | Ossifying mass                      |
| 34   | Serous effusion                     |
| 35   | Fibrinous-purulent effusion         |
| 36   | Hemorrhagic effusion                |

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Table 13. Codes of microscopic changes.

| Code | Change | Code | Change |
|------|--------|------|--------|
| 1    | No changes | 57  | Fibroangiomatosis |
| 2    | Mild regressive changes | 58  | Simple polyp |
| 3    | Serious regressive changes | 59  | Polyp with cellular distypias |
| 4    | Necrosis | 60  | Papilloma |
| 5    | Ulcer | 61  | Fibropapilloma |
| 6    | Amyloidosis | 62  | Acanthoma |
| 7    | Hyalinosis | 63  | Trichoepithelioma |
| 8    | Colloid-cystic degeneration | 64  | Simple adenoma |
| 9    | Calcifications | 65  | Muciparous adenoma |
| 10   | Emphysema | 66  | Colloid-cystic adenoma |
| 11   | Vascular changes (hyperemia, dilatation of sinusoids and other vessels, edema and hemorrhage) | 67  | Exocrine pancreas adenoma |
| 12   | Hematocyst | 68  | Endocrine pancreas adenoma (islet cell adenoma) |
| 13   | Organized fibrinous coagulum | 69  | Chromophobe adenoma |
| 14   | Hemorrhagic effusion | 70  | Chromophilic adenoma |
| 15   | Acute phlogistic changes (including abscess) | 71  | Cortical adenoma |
| 16   | Chronic phlogistic changes (also reactive) | 72  | Medullary adenoma |
| 17   | Particular granulomatous changes | 73  | Cholangioma |
| 18   | Phlogistic effusion | 74  | Hepatocellular adenoma or hepatoma |
| 19   | Thickening of capsule | 75  | Tumor of granulosa and of theca |
| 20   | Thickening of submesothelial tissues | 76  | Leydig cell tumor |
| 21   | Fibrosis | 77  | Other epithelial benign tumors |
| 22   | Post necrotic fibrosis (comprehensive of cirrhosis) | 78  | Fibroma |
| 23   | Fibrous thickening of vessels | 79  | Mixoma |
| 24   | Cystic ectasia of blood vessels with fibrosis | 80  | Lipoma |
| 25   | Cystic ectasia of blood vessels with fibrosis and hyperplasia of perithelial cells | 81  | Leiomyoma |
| 26   | Cystic ectasia of blood vessels with fibrosis and dysplasia of perithelial cells | 82  | Rhabdomyoma |
| 27   | Cellular depletion and atrophy (with or without fibrosis) | 83  | Chondroma |
| 28   | Simple cyst | 84  | Osteoma |
| 29   | Hemorrhagic cyst | 85  | Angioma |
| 30   | Multiple simple cyst | 86  | Fibroangiomia |
| 31   | Multiple hemorrhagic cyst | 87  | Ossifying angioma |
| 32   | Dilatation of organs with cavity (including hydropneumothorax) | 88  | Other benign tumors of connective tissue |
| 33   | Hyperplasia and squamous metaplasia | 89  | Fibroadenoma |
| 34   | Glandular simple and cystic hyperplasia | 90  | Adenomyoma |
| 35   | Diffused parenchymal hyperplasia | 91  | Benign tumors of nervous ganglia (ganglioneuroma) and benign sympathetic tumors of adrenal medulla |
| 36   | Nodular parenchymal hyperplasia | 92  | Benign tumors of peripheral nerves (neurilemoma) |
| 37   | Cortical hyperplasia | 93  | Carcinoma |
| 38   | Medullary hyperplasia | 94  | Carcinoma with metastases |
| 39   | Hyperplasia of stroma | 95  | Basocellular carcinoma |
| 40   | Reactive hyperplasia | 96  | Basocellular carcinoma with metastases |
| 41   | Simple proliferation of lymphoreticular cells with myelopoesis | 97  | Squamouscellular carcinoma |
| 42   | Proliferation of angioblastic cells | 98  | Squamouscellular carcinoma with metastases |
| 43   | Fibroangiolastic proliferation | 99  | Transitional cell carcinoma |
| 44   | Proliferation of lipocytes | 100 | Transitional cell carcinoma with metastases |
| 45   | Proliferation of biliary ducts | 101 | Adenocarcinoma |
| 46   | Proliferation of renal tubules and/or of nephroblastema | 102 | Adenocarcinoma with metastases |
| 47   | Atypical hyperplasia | 103 | Biliary duct adenocarcinoma |
| 48   | Cholangiofibrosis | 104 | Biliary duct adenocarcinoma with metastases |
| 49   | Dysplasia (comprehensive of neoplastic parenchymal nodule of liver) | 105 | Hepatocellular carcinoma or hepatocarcinoma |
| 50   | Simple and cystic glandular dysplasia | 106 | Hepatocarcinoma with metastases |
| 51   | Cortical dysplasia | 107 | Exocrine pancreas adenocarcinoma |
| 52   | Medullary dysplasia | 108 | Exocrine pancreas adenocarcinoma with metastases |
| 53   | Dysplasia of angioblastic cells | 109 | Cortical adenocarcinoma |
| 54   | Papillomatosis | 110 | Cortical adenocarcinoma with metastases |
| 55   | Acanthomatosis | 111 | Pheochromoblastoma |
| 56   | Angiomatosis | 112 | Pheochromoblastoma with metastases |
|      |     | 113 | Nephroblastoma |
|      |     | 114 | Nephroblastoma with metastases |
|      |     | 115 | Seminoma |
|      |     | 116 | Seminoma with metastases |
|      |     | 117 | Melanoma |
|      |     | 118 | Melanoma with metastases |
|      |     | 119 | Other malignant epithelial tumors |

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For each animal an individual final card was prepared, which included data on experimental factors, survival, weight at 6, 12, 18 and 24 months, and any gross and microscopic lesions. Samples are shown in Figures 1 and 2.

**Presentation of Pathological Data.** The results of all VC experiments, as well as those of any other experiment performed in our laboratory, will be presented in the final report (now in press) with the same types of tables, in the same sequence.

This type of presentation has been made possible by the knowledge of the basic pathology of the animal used, which enabled us to make an approximated census of the expected lesions.

Such a procedure permits a quick comparison among the results of different experiments of the same project and possibly of the results of projects studying different compounds.

**Interpretation of the Data.** The data were subjected to statistical analysis. Although statistical analysis provides an extremely important tool for interpreting the meaning of the results of long-term bioassays, it should be stressed that there may be smaller differences between exposed and control groups which do not reach statistical significance, while these differences could still have meaning from an oncological point of view (particularly in the case of tumors which are infrequent in the animal colony).

Therefore, the most important data should be commented on both in the light of the statistical analysis performed and from a biological point of view.

The methodological protocol adopted meets the requirements of the recent Good Laboratory Practice Act.

**Results**

Part of these results, namely those dealing with seven basic experiments on the effects of long-term exposure to a range of 14 doses by inhalation (from 30,000 to 1 ppm) and of six doses by ingestion (from 50 mg to 0.03 mg/kg bw), on Sprague-Dawley rates, were presented previously (1, 2).

A report, for limited circulation, dealing with part of the results has also appeared (3).

The results of the whole project, with detailed tables, will appear in a monograph which will encompass data on survival rate, body weight, regressive and inflammatory changes, benign and malignant tumors, neoplastic precursors, and the most important proliferative changes.

With this report we are presenting only tables summarizing the most outstanding results and information, and what we do believe to be the integrative documentation and strictly necessary comments.

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Tables 15-31 presented data on the incidence of the tumors which have been considered as dependent or possibly correlated to VC exposure, in 17 different experiments on the effects of VC in different animal systems, by different routes, at different doses and with various schedules of treatment. Explanations of abbreviations used in the tables are given in Table 14.

The possible leukemogenic effect of VC in golden hamsters is expressed both by the slight increase in incidence but more by the decrease in latency time (from 16 weeks in animals treated at 10,000 ppm to 36 weeks in control animals).

Examples of the most characteristic microscopic features of these tumors were given in a previous publication (4).

The data on dose-response relationship in long-term treatment experiments, by inhalation and by ingestion, in Sprague-Dawley rats, Wistar rats and Swiss mice, with reference to the incidence of total malignant and benign tumors, and the most important neoplasias observed, are shown in Tables 32-63.

The striking effect of the influence of scheduled treatment is pointed out by the results shown in Table 64.

Examples of the marked influence of the animals used in determining the neoplastic response are shown in Tables 65-67, which point out the effects of species, strain and age.

**Conclusions**

VC-dependent tumors are identified on the basis of one or more of the following parameters: (a) sharply enhanced incidence; (b) rare or exceptional occurrence in the colony of the animal used; (c) dose-response relationship; (d) association of precursor lesions.

From the presented data the following conclusions may be drawn.

1. VC causes tumors in all the different animal systems tested.
2. VC is a multipotential carcinogen, since it causes tumors of different types in different sites (Table 68).
3. Some types of tumors are observed in all the animals studied, i.e., liver angiosarcoma, whereas others are observed in only one animal system.
4. The degree of evidence of correlation between VC treatment and the tumors considered as VC-dependent varies from tumor to tumor.
5. VC shows carcinogenic effects both when given by inhalation and ingestion and possibly by injection.
6. Both through inhalation and ingestion experiments there is a clear-cut dose-response relationship.
7. The duration of treatment and schedule of treatment greatly affects the neoplastic response.
8. The neoplastic response, in qualitative and quantitative terms, is greatly affected by the species, the strain and the sex of the animals studied.
9. Newborn animals appear to be extremely responsive and easily develop liver tumors, both hepatocarcinomas and angiosarcomas.
10. VC produces carcinogenic effects on embryos via the placenta.
11. With the above criteria for identifying VC-dependent tumors, VC shows carcinogenic effects even at low doses, namely down to 50 ppm and less.
12. The results of the seven basic experiments studying the effects of doses of VC as given by inhalation (BT1, 2, 6, 9, 15), and ingestion (BT11, 27), have been subject to statistical analysis following the Fisher exact probability test (p<0.05). The total cancer-bearing animals and the tumors...
Macroscopic changes:

| Site                              | Type                        | Side | No. | Code   |
|-----------------------------------|-----------------------------|------|-----|--------|
| Subcutaneous tissues              | Hemorrhagic nodule          |      | 1   | 4, 28, D1 |
| Lung                              | Hemorrhagic nodule          |      | 1   | 11, 28, D12 |
| Pleura and pleural cavity         | Hemorrhagic effusion        |      | 12  | 6      |
| Forestomach                       | No changes                  |      | 14  | 1      |
| Liver                             | Hemorrhage                  |      | 17  | 6      |
| Peritoneum and peritoneal cavity  | No changes                  |      | 19  | 1      |
| Adrenals                          | Hemorrhagic mass            | Sn   | 1   | 32, 32, C1, D1 |
| Harderian glands                  | No changes                  | Sn   | 39  | 1, C1  |
| Intrathoracic and parathymic      | In toto enlargement         |      | 51  | 17     |
| lymph node                        |                             |      |     |        |

Microscopic changes:

| Site                              | Type                        | Side | No. | Code   |
|-----------------------------------|-----------------------------|------|-----|--------|
| Subcutaneous tissues              | Fibroangioma                |      | 4   | 86     |
| Lung                              | Secondary neoplastic        |      | 11  | 158    |
|                                  | localization               |      |     |        |
|                                  | (liver angiosarcoma)        |      | (17, 138) | |
| Pleura and pleural cavity        | Secondary neoplastic        |      | 12  | 158    |
|                                  | localization               |      |     |        |
|                                  | (liver angiosarcoma)        |      | (17, 138) | |
| Forestomach                       | Papilloma                   |      | 14  | 60     |
| Liver                             | Hepatocarcinoma            |      | 17  | 105    |
|                                  | Angiosarcoma with metastases|      | 17  | 138    |
| Peritoneum and                    | Secondary neoplastic        |      | 19  | 158    |
| peritoneal cavity                 | localization               |      |     |        |
|                                  | (liver angiosarcoma)        |      | (17, 138) | |
| Adrenals                          | Cortical adenoma           | Sn   | 32  | 71, C1 |
| Harderian glands                  | Abscess                     | Sn   | 39  | 15, C1 |
| Intrathoracic and parathymic      | No changes                  | Sn   | 51  | 1      |
| lymph node                        |                             |      |     |        |

Figure 2. Sample record of macroscopic and microscopic changes.

Table 14. Abbreviations used in tables.

| Abbreviation | Description                                      |
|--------------|--------------------------------------------------|
| T            | Tumor                                            |
| Ca           | Carcinoma                                        |
| Ep T         | Epithelioma                                      |
| Pa           | Papilloma                                        |
| Ac           | Acanthoma                                        |
| Ad           | Adenoma                                          |
| Ad †         | Adenoma in malignant transformation              |
| MT           | Malignant tumors (total if not otherwise specified) |
| BT           | Benign tumors (total if not otherwise specified)  |
| LAS          | Liver angiosarcoma                               |
| LA           | Liver angiomat                                   |
| ELAS         | Extra-liver angiosarcoma                         |
| ELA          | Extra-liver angioma                              |
| Nephro-BL    | Nephroblastoma                                   |
| Neuro-BL     | Neuroblastoma                                    |
| A            | Angioblastic hyperplasia in liver                |
| A †          | Angioblastic dysplasia in liver                  |
| Neop. nod.   | Neoplastic nodules of liver                      |
| Nod. hyp.    | Nodular hyperplasia of liver                     |
| Dif. hyp.    | Diffused hyperplasia of liver                    |
| + +          | Marked                                           |
| + + +        | Very marked                                      |

The incidence of total malignant and benign tumours is given as the total number of tumors per 100 animals (one animal may bear more than one malignant or benign tumor) on the basis of the tumors observed among the animals alive, when the first tumor was observed in the experiment.

The incidence of specific tumour is given, as percent of the animals bearing the tumor considered, referred to the animals alive when the first tumor was observed (in parentheses).

Significantly in excess in these experiments, in relation to dose, are given in Tables 69 and 70.

The Fisher exact probability test at 95% confidence is, in relation to the above, not "sensitive" enough, in our experimental conditions.

Biologically, in our opinion, the following results, although not statistically significant according to the test used, should be given proper attention.

Extrahepatic angiosarcomas of different sites are observed at a very low incidence dose in untreated Sprague-Dawley rats of our colony. Results of experiments BT1 and particularly BT9, however, strongly suggest a relationship between these tumors and VC exposure. This relationship is supported by the excessive incidence of extrahepatic vascular tumors in mice treated with VC (BT4).

Few cases of hepatomas have been observed in treated groups, particularly in BT1. This tumor is exceptionally rare in our colony of animals, and none have been observed in the control group of the 17 experiments. Moreover the relationship with treatment is supported by the fact that a high incidence of hepatomas has been observed in Sprague-Dawley rats, following neonatal exposure to a high dose for a short period (BT14).

In view of their rareness or nonobservation in the colony of animal used, for the following tumors it should be stressed that attention should be paid to

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Table 15. Experiment BT1.

| Group and concentration | Tumors/100 animals |  |  |  |  |  |  |  |  |  |
|-------------------------|--------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                         | MT    | BT    | LAS | LA  | ELAS | ELA | Hepatomas | Nephro- | Neuro- | Zymbal Gl.Ca | Skin EpT | Fore-stomach | Mammary |
|                         |       |       |     |     |      |     |           | BL     | BL    |            |          | Pa&Ac          | MT     |
| I 10,000 ppm            | 81.7  | 23.3  | 11.7| 5.0 | 5.0  | 1.7 | 8.3       | 11.7   | 5.0   | 11.7        | 5.0     | –              | 5.0    |
| II 6000 ppm             | 60.0  | 38.3  | 22.0| 3.4 | 5.1  | 1.7 | 8.5       | 5.1    | 11.9  | 3.4         | 1.7     | –              | –      |
| III 2500 ppm            | 63.3  | 20.0  | 21.7| 5.0 | 5.0  | 1.7 | 10.0      | 6.7    | 3.3   | 1.7         | –        | 3.3            | –      |
| IV 500 ppm              | 51.7  | 13.3  | 10.0| 1.7 | 1.7  | 8.3 | 10.0      | 6.7    | 1.7   | –           | 1.7     | –              | 1.7    |
| V 150 ppm               | 30.0  | 25.0  | 5.1 | 1.7 | 3.4  | –   | 1.7       | 8.5    | –     | 3.4         | –        | 3.4            | –      |
| VI 50 ppm               | 15.0  | 36.7  | 1.7 | 1.7 | 3.3  | –   | 1.7       | –      | 1.7   | 1.7         | 3.3      | –              | –      |
| VII No treatment (control) | 13.3 | 43.3  | –   | –   | 3.4  | –   | –         | –      | –     | 1.7         | –        | –              | –      |

*Exposure by inhalation to VC in air at 10,000, 6000, 2500, 500, 250, and 50 ppm; 4 hr/day, 5 days/week, for 52 weeks. Sprague-Dawley rats, M and F, 13 weeks old. Results after 135 weeks (end of experiment).

Table 16. Experiment BT2.

| Group and concentration | Tumors/100 animals |  |  |  |  |  |  |  |  |  |  |  |  |
|-------------------------|--------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                         | MT    | BT    | LAS | LA  | ELAS | ELA | Hepatomas | Nephro- | Neuro- | Zymbal Gl.Ca | Skin EpT | Fore-stomach | Mammary |
|                         |       |       |     |     |      |     |           | BL     | BL    |            |          | Pa&Ac          | MT     |
| I 200 ppm               | 35.0  | 21.7  | 10.0| 3.3 | 0.8  | 0.8 | 2.5       | 5.8    | –     | 3.3        | 4.2     | –              | 5.0    |
| II 150 ppm              | 35.0  | 25.0  | 5.0 | –   | –    | 0.8 | 2.5       | 9.2    | –     | 3.4        | 3.4     | 1.7            | 5.0    |
| III 100 ppm             | 21.7  | 27.5  | 0.8 | 0.8 | –    | –   | 8.3       | –      | 0.8   | 3.3        | 3.3     | 3.3            | –      |
| IV No treatment (control) | 15.7 | 21.6  | –   | –   | 1.1  | –   | –         | –      | –     | 1.1        | 1.6     | 1.0            | –      |

*Exposure by inhalation to VC in air at 200, 150, 100 ppm; 4 hr/day, 5 days/week, for 52 weeks. Sprague-Dawley rats, M and F, 13 weeks old. Results after 143 weeks (end of experiment).

Table 17. Experiment BT6.

| Group and concentration | Tumors/100 animals |  |  |  |  |  |  |  |  |  |  |  |  |  |
|-------------------------|--------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                         | MT    | BT    | LAS | LA  | ELAS | ELA | Hepatomas | Nephro- | Neuro- | Zymbal Gl.Ca | Skin EpT | Fore-stomach | Mammary |
|                         |       |       |     |     |      |     |           | BL     | BL    |            |          | Pa&Ac          | MT     |
| I 30,000 ppm            | 100.0 | 50.0  | 30.0| 1.7 | 1.7  | 5.0 | 1.7       | 1.7    | 1.7   | 58.3       | 1.7     | 18.3          | 3.3    |

*Exposure by inhalation to VC in air at 30,000 ppm; 4 hr/day, 5 days/week, for 52 weeks. Sprague-Dawley rats, M and F, 17 weeks old. Results after 68 weeks (end of experiment).
Table 18. Experiment BT9.*

| Group and concentration | Tumors/100 animals | Animals with tumors, % | Fore- stomach Pa&Ac | Mammary MT |
|-------------------------|--------------------|------------------------|---------------------|------------|
| MT | BT | LAS | LA | ELAS | ELA | Hepatomas | Nephro- | Neuro- | Zymbal | Skin | EpT |
| I  | 50 ppm | No treatment | (control) | |

*Exposure by inhalation to VC in air at 50 ppm; 4 hr/day, 5 days/week, for 52 weeks. Sprague-Dawley rats, M and F, 13 weeks old. Results after 142 weeks (end of experiment).

Table 19. Experiment BT15.*

| Group and concentration | Tumors/100 animals | Animals with tumors, % | Fore- stomach Pa&Ac | Mammary MT |
|-------------------------|--------------------|------------------------|---------------------|------------|
| MT | BT | LAS | LA | ELAS | ELA | Hepatomas | Nephro- | Neuro- | Zymbal | Skin | EpT |
| I  | 25 ppm | II | 10 ppm | III | 5 ppm | IV | 1 ppm | V  | No treatment | (control) | |

*Exposure by inhalation to VC in air at 25, 10, 5, 1 ppm; 4 hr/day, 5 days/week, for 52 weeks. Sprague-Dawley rats, M and F, 13 weeks old. Results after 147 weeks (end of experiment).

Table 20. Experiment BT3.*

| Group and concentration | Tumors/100 animals | Animals with tumors, % | Fore- stomach Pa&Ac | Mammary MT |
|-------------------------|--------------------|------------------------|---------------------|------------|
| MT | BT | LAS | LA | ELAS | ELA | Hepatomas | Nephro- | Neuro- | Zymbal | Skin | EpT |
| I  | 10,000 ppm | II | 6000 ppm | III | 2500 ppm | IV | 500 ppm | V  | 250 ppm | VI | 50 ppm | VII | No treatment | (control) | |

*Exposure by inhalation to VC in air at 10,000, 6000, 2500, 500, 250, and 50 ppm; 4 hr/day, 5 days/week, for 17 weeks. Sprague-Dawley rats, M and F, 12 weeks old. Results after 156 weeks (end of experiment).

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Table 21. Experiment BT10.*

| Group and concentration | Tumors/100 animals | Animals with tumors, % | Fore-stomach Pa&Ac | Mammary MT |
|-------------------------|-------------------|------------------------|--------------------|------------|
|                         | MT                | BT                     | LAS LA ELAS ELA Hepatomas Nephro- Neuro- Zymbal Gl.Ca Skin EpT |               |
| I 10,000 ppm            | 33.3              | 41.7                   | 0.8               | 0.8         | 7.6        | 2.5        | 11.0       |
| II 6000 ppm             | 30.0              | 45.0                   | 0.8               | 1.7         | 7.5        | 1.7        | 10.8       |
| III 10,000 ppm          | 35.8              | 45.0                   | 0.8               | 0.8         | 7.5        | 2.5        | 13.4       |
| IV 6000 ppm             | 30.8              | 39.2                   | 2.5               | 1.7         | 4.2        | 3.4        | 9.3        |
| V 10,000 ppm            | 41.7              | 45.0                   | 0.8               | 0.8         | 7.5        | 1.7        | 10.8       |
| VI 6000 ppm             | 33.3              | 50.8                   | 0.8               | 1.7         | 7.5        | 2.5        | 10.0       |
| VII No treatment (control) | 16.6          | 41.0                   | 0.4               |             |            | 0.9        | 2.2        | 7.5       |

*Exposure by inhalation to VC in air at 10,000, 6000, ppm; 4 hr/day, 5 days/week, for 5 weeks (groups I and II) or 1 hr/day, 4 days/week, for 25 weeks (groups III and IV) or 4 hr/day, once weekly, for 25 weeks (groups V and VI) (100 hr). Sprague-Dawley rats, M and F, 19 weeks old. Results after 154 weeks (end of experiment).

Table 22. Experiment BT5.*

| Group and concentration | Tumors/100 animals | Animals with tumors, % | Fore-stomach Pa&Ac | Mammary MT |
|-------------------------|-------------------|------------------------|--------------------|------------|
|                         | MT                | BT                     | LAS LA ELAS ELA Hepatomas Nephro- Neuro- Zymbal Gl.Ca Skin EpT |               |
| I 10,000 ppm            | 6.7               | 36.7                   | -                  | -          | -          | 3.3        | -          |
| II 6000 ppm             | 6.7               | 23.3                   | -                  | -          | -          | -          | -          |
| III 10,000 ppm          | 29.6              | 22.2                   | -                  | -          | -          | 5.9        | 9.8        | 2.0        | 2.0       |
| IV 6000 ppm             | 21.9              | 46.9                   | -                  | 3.1        | -          | 9.4        | 3.1        | 3.1        | 6.2       |

*Exposure by inhalation to VC in air at 10,000, 6000 ppm of breeders; 4 hr/day for 1 week (from 12th to 18th day of pregnancy). Sprague-Dawley rats, M and F, 19 weeks old (breeders). Breeders (groups I and II) and offsprings (groups III and IV). Results after 143 weeks (end of experiment).

Table 23. Experiment BT14.*

| Group and concentration | Tumors/100 animals | Animals with tumors, % | Fore-stomach Pa&Ac | Mammary MT |
|-------------------------|-------------------|------------------------|--------------------|------------|
|                         | MT                | BT                     | LAS LA ELAS ELA Hepatomas Nephro- Neuro- Zymbal Gl.Ca Skin EpT |               |
| I 10,000 ppm (breeders) | 16.7              | 66.7                   | -                  | -          | -          | -          | -          |
| II 6000 ppm (breeders)  | -                 | -                      | -                  | -          | -          | -          | -          |
| III 10,000 ppm (newborn)| 100.0             | 55.5                   | 34.1               | 6.8        | 45.4       | 2.3        | 2.3        | -          |
| IV 6000 ppm (newborn)   | 109.3             | 58.1                   | 40.5               | 2.4        | 4.8        | 4.8        | 2.4        | -          |

*Exposure by inhalation to VC in air at 10,000 and 6000 ppm, 4 hr/day, 5 days/week, for 5 weeks (from 1 day to 5 weeks of age). Sprague-Dawley rats, M and F, 21 weeks old (breeders) (groups I and II) and newborn (groups III and IV). Results after 124 weeks (end of experiment).
Table 24. Experiment BT7.*

| Group and concentration | Tumors/100 animals | Animals with tumors, % |
|-------------------------|--------------------|------------------------|
|                         | MT | BT | LAS | LA | ELAS | ELA | Hepatomas | NephroBL | NeuroBL | Zymbal | Gl.Ca | EpT | Pa&Ac | Fore-stomach | Skin | stomach |
| I 10,000 ppm             | 50.0 | 10.0 | 29.6 | - | - | - | 3.7 | 11.1 | 7.4 | - | - | - | - | - |
| II 6000 ppm              | 53.3 | 20.0 | 11.5 | 7.7 | 3.8 | 3.8 | 7.7 | 7.7 | 3.8 | 7.7 | - | - | - | - | - |
| III 2500 ppm             | 26.7 | 13.3 | 12.0 | - | 4.0 | - | 4.0 | - | 4.0 | - | - | - | - | - | - |
| IV 500 ppm               | 30.0 | 10.0 | 10.7 | 3.6 | - | - | 7.1 | - | - | - | - | - | - | - | - |
| V 250 ppm                | 13.3 | 16.7 | 3.7 | - | 3.7 | 3.7 | - | - | - | 3.7 | - | - | - | - | - |
| VI 50 ppm                | 16.7 | 6.7 | - | 3.6 | - | - | - | - | - | - | - | - | - | - | - |
| No treatment (control)   | 15.0 | 15.0 | - | 2.6 | 2.6 | - | - | - | - | - | - | - | - | - | - |

*Exposure by inhalation to VC in air at 10,000, 6000, 2500, 500, 250, and 50 ppm; 4 hr/day, 5 days/week, for 52 weeks. Wistar rats, M, 11 weeks old. Results after 165 weeks (end of experiment).

Table 25. Experiment BT17.*

| Group and concentration | Tumors/100 animals | Animals with tumors, % |
|-------------------------|--------------------|------------------------|
|                         | MT | BT | LAS | LA | ELAS | ELA | Hepatomas | NephroBL | NeuroBL | Zymbal | Gl.Ca | EpT | Pa&Ac | Fore-stomach | Skin | stomach |
| I 1 ppm                 | 24.2 | 29.2 | - | 1.0 | 3.0 | 5.0 | 1.0 | - | - | 2.0 | - | - | - | - | - |
| II No treatment (control) | 20.0 | 18.5 | - | - | - | - | - | - | - | 3.2 | - | - | - | - | - |

*Exposure by inhalation to VC in air at 1 ppm; 4 hr/day, 5 days/week, for 52 weeks. Wistar rats, M, 13 weeks old. Results after 134 weeks (end of experiment).

Table 26. Experiment BT4.*

| Group and concentration | Tumors/100 animals | Animals with tumors, % |
|-------------------------|--------------------|------------------------|
|                         | MT | BT | LAS | LA | ELAS | ELA | Lung T | Mammary | Skin | Fore-stomach | Ca | EpT | Pa&Ac |
| I 10,000 ppm            | 50.0 | 98.3 | 17.8 | 10.7 | 1.8 | 7.1 | 82.1 | 23.2 | 7.1 | 1.8 | - | - | - |
| II 6000 ppm             | 56.7 | 100.0 | 21.7 | 11.7 | 1.7 | 5.0 | 78.3 | 13.3 | 11.7 | 1.7 | - | - | - |
| III 2500 ppm            | 58.3 | 90.0 | 27.1 | 8.5 | 13.5 | 1.7 | 67.8 | 13.5 | 6.8 | 1.7 | - | - | - |
| IV 500 ppm              | 58.3 | 103.3 | 23.3 | 8.3 | 11.7 | 5.0 | 83.3 | 13.3 | 3.3 | - | - | - | - |
| V 250 ppm               | 63.3 | 98.3 | 30.0 | 18.3 | 5.0 | 5.0 | 68.3 | 20.0 | 1.7 | 1.7 | - | - | - |
| VI 50 ppm               | 28.3 | 23.3 | 2.3 | 2.7 | 7.7 | 3.3 | 10.0 | 20.0 | 1.7 | 1.7 | - | - | - |
| VII No treatment (control) | 14.7 | 14.7 | - | - | 0.7 | 0.7 | 10.0 | 0.7 | 1.3 | - | - | - | - |

*Exposure by inhalation to VC in air at 10,000, 6000, 2500, 500, 250, and 50 ppm; 4 hr/day, 5 days/week, for 30 weeks. Swiss mice, M and F, 11 weeks old. Results after 81 weeks (end of experiment).
### Table 27. Experiment BT8.a

| Group and concentration | Tumors/100 animals | Animals with tumors, % | Acoustic Duct EpT | Skin EpT | Metastases | Fore-stomach Leukae-miasb |
|-------------------------|--------------------|------------------------|-------------------|---------|------------|--------------------------|
|                         | MT     | BT     | LAS    | LA   | ELA    | Hepato-mas   | Cholan-gio-Ca | Cholan-giomas | BlOmegas | GLomas | Mammary |
| I 10,000 ppm            | 50.0   | 73.3   | 3.3    | 6.7  | (2/30) | 6.7         | 13.3         | 3.3          | 23.3      | 3.3      | 33.3     | 16.7 |
| II 6000 ppm             | 40.0   | 63.3   | 3.3    | 3.3  | (1/30) | 3.3         | 16.7         | 6.7          | 3.3       | 6.7      | 33.3     | 20.0 |
| III 2500 ppm            | 43.3   | 103.3  | 6.7    | 6.7  | (2/30) | 26.7        | 10.0         | 10.0         | 30.0      | 56.7     | 30.0     |
| IV 500 ppm              | 53.3   | 63.3   | 3.3    | 6.7  | (1/30) | 20.0        | 10.0         | 23.3         | 30.0      | 16.7     | 30.0     |
| V                        | 30.0   | 43.3   | 6.7    | 6.7  | (1/30) | 23.3        | 30.0         | 3.3          | 10.0      | 20.0     | 30.0     |
| VI 2500 ppm             | 50.0   | 40.0   | 6.7    | 6.7  | (1/30) | 20.0        | 10.0         | 3.3          | 10.0      | 20.0     | 30.0     |
| VII No treatment (control) | 20.0  | 46.7   | 6.7    | 6.7  | (1/30) | 36.7        | 5.0          | 5.0          | 13.3      | 30.0     | 30.0     |

*aExposure by inhalation to VC in air at 10,000, 6000, 2500, 500, 250, and 50 ppm; 4 hr/day, 5 days/week, for 30 weeks. Golden hamsters, M, 11 weeks old. Results after 109 weeks (end of experiment).*

*bLatency time in weeks: Group I, 16.7; Group II, 27.2; Group III, 30.8; Group IV, 19.0; Group V, 22.5; Group VI, 35.3; Group VII, 36.5.

### Table 28. Experiment BT11.a

| Group and concentration | Tumors/100 animals | Animals with tumors, % | Acoustic Duct EpT | Skin EpT | Mammary |
|-------------------------|--------------------|------------------------|-------------------|---------|--------|
|                         | MT     | BT     | LAS    | LA   | ELA    | Hepato-mas | Nephro-bl | Neuro-bl | Gl.Ca   | BlOmegas |
| I 50.00 mg/kg           | 38.7   | 35.0   | 21.2   | 3.7  | 2.5    | 25.0       | 1.2       | 25.0      | 1.2      | 25.0     | 5.0     |
| II 16.65 mg/kg          | 50.0   | 43.3   | 12.5   | 3.3  | 3.7    | 25.0       | 25.0      | 25.0      | 25.0     | 25.0     | 5.0     |
| III 3.33 mg/kg          | 10.0   | 25.0   | 25.0   | 2.5  | 1.2    | 25.0       | 25.0      | 25.0      | 25.0     | 25.0     | 5.0     |
| IV Olive oil (control)  | 13.7   | 22.5   | 6.7    | 6.7  | 6.7    | 25.0       | 25.0      | 25.0      | 25.0     | 25.0     | 5.0     |

*aExposure by ingestion (stomach tube) of VC in olive oil at 50.00, 16.65 and 3.33 mg/kg body weight, once daily, 4-5 days/week, for 52 weeks. Sprague-Dawley rats, M and F, 13 weeks old. Results after 136 weeks (end of experiment).*

### Table 29. Experiment BT27.a

| Group and concentration | Tumors/100 animals | Animals with tumors, % | Acoustic Duct EpT | Skin EpT | Mammary |
|-------------------------|--------------------|------------------------|-------------------|---------|--------|
|                         | MT     | BT     | LAS    | LA   | ELA    | Hepato-mas | Nephro-bl | Neuro-bl | Gl.Ca   | BlOmegas |
| I 1.0 mg/kg             | 24.7   | 35.3   | 2.0    | 0.7  | 0.7    | 3.3        | 3.3       | 3.3       | 2.0      | 1.3     | 8.0     |
| II 0.3 mg/kg            | 13.3   | 28.0   | 0.7    | 0.7  | 0.7    | 3.3        | 1.3       | 1.3       | 2.7      | 2.7     | 1.3     |
| III 0.03 mg/kg          | 18.0   | 31.3   | 0.7    | 0.7  | 0.7    | 3.3        | 0.7       | 0.7       | 3.3      | 3.3     | 1.3     |
| IV Olive oil (control)  | 16.0   | 28.7   | 0.7    | 0.7  | 0.7    | 3.3        | 0.7       | 0.7       | 3.3      | 3.3     | 1.3     |

*aExposure by ingestion (stomach tube) of VC in olive oil at 1.0, 0.3, 0.03 mg/kg body weight, once daily, 4-5 days/week, for 59 weeks. Sprague-Dawley rats, M and F, 10 weeks old. Results after 136 weeks (end of experiment).*

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Table 30. Experiment BT12.*

| Group and dose | Tumors/100 animals | Animals with tumors, % |
|---------------|--------------------|-----------------------|
|               | MT | BT | LAS | LA | ELAS | ELA | Hepatomas | Nephro- | Neuro- | Zymbal | Skin EpT | Fore- | Mammary |
| I 4.25 mg x 4 | 13.8 | 25.0 | - | - | - | - | - | - | - | - | - | 1.8 | 1.8 |
| II 4.25 mg x 3 | 16.7 | 28.3 | - | - | - | - | - | - | - | - | - | 1.9 | 1.9 |
| III 4.25 mg x 2 | 11.7 | 18.3 | - | - | - | 1.8 | - | - | - | - | 1.8 | 3.6 | 3.6 |
| IV 4.25 mg x 1 | 20.0 | 35.0 | - | - | - | - | - | - | - | - | - | 3.6 | 3.6 |
| V Olive oil (control) | 8.3 | 31.7 | - | - | - | - | - | - | - | - | - | 3.6 | 3.6 |

*Exposure by intraperitoneal injection of VC, 4.25 mg in olive oil (1 ml), 4, 3, 2 times, at two month intervals or once only. Sprague-Dawley rats, M and F, 17 weeks old. Results after 144 weeks (end of experiment).

Table 31. Experiment BT13.*

| Group and dose | Tumors/100 animals | Animals with tumors, % |
|---------------|--------------------|-----------------------|
|               | MT | BT | LAS | LA | ELAS | ELA | Hepatomas | Nephro- | Neuro- | Zymbal | Skin EpT | Fore- | Mammary |
| I 4.25 mg | 16.0 | 17.3 | - | - | - | - | 1.3 | - | - | - | - | 4.0 | 4.0 |
| II Olive oil (control) | 13.3 | 26.7 | - | - | - | 1.3 | - | - | - | - | 1.3 | 1.3 |

*Exposure by subcutaneous injection of VC, 4.25 mg, in olive oil (1 ml), single dose. Sprague-Dawley rats, M and F, 21 weeks old. Results after 145 weeks (end of experiment).

Table 32. Incidence of total MT and BT in Sprague-Dawley rats, in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiments | Concentration (ppm) | Tumors/100 animals |
|-------------|---------------------|--------------------|
|             | MT | F | Total | MT | F | Total |
| BT 6        | 30,000 | 76.7 | 123.3 | 100.0 | 40.0 | 60.0 | 50.0 |
| BT 1        | 10,000 | 80.0 | 83.3 | 81.7 | 26.7 | 20.0 | 23.3 |
|             | 6,000 | 46.7 | 73.3 | 60.0 | 13.3 | 63.3 | 38.3 |
|             | 2,500 | 53.3 | 73.3 | 63.3 | 16.7 | 23.3 | 20.0 |
|             | 500  | 23.3 | 80.0 | 51.7 | 13.3 | 13.3 | 13.3 |
|             | 250  | 23.3 | 36.7 | 30.0 | 23.3 | 26.7 | 25.0 |
|             | 100  | 40.0 | 30.0 | 35.0 | 10.0 | 33.3 | 21.7 |
| BT 2        | 200  | 40.0 | 48.3 | 35.0 | 21.7 | 28.3 | 25.0 |
|             | 150  | 21.7 | 20.0 | 21.7 | 20.0 | 35.0 | 27.5 |
|             | 100  | 23.3 | 20.0 | 21.7 | 20.0 | 35.0 | 27.5 |
|             | 50   | 6.7  | 23.3 | 15.0 | 23.3 | 50.0 | 36.7 |
| BT 1        | 50   | 20.7 | 68.0 | 44.3 | 23.3 | 60.0 | 41.7 |
| BT 9        | 25   | 20.0 | 46.7 | 33.3 | 31.7 | 65.0 | 58.3 |
| BT 15       | 10   | 18.3 | 45.0 | 31.7 | 30.0 | 76.7 | 53.3 |
|             | 5    | 25.0 | 46.7 | 35.8 | 25.3 | 81.7 | 55.0 |
|             | 1    | 15.0 | 30.0 | 22.5 | 18.3 | 70.0 | 44.2 |

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Table 33. Incidence of LAS, LA, A++/+ + + ↑ and A++/+ + + in Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiments | Concentration, ppm | Animals with tumors and correlated changes, % |
|-------------|-------------------|---------------------------------------------|
|             |                   | LAS  | LA  | A++/+ + + ↑ | A++/+ + + |
|             | M     F     Total | M     F     Total | M     F     Total | M     F     Total | M     F     Total | M     F     Total |
| BT 6        | 30,000 | 16.6 43.3 30.0 | 3.3 1.7 | 6.7 6.7 6.7 | 6.7 13.3 10.0 |
| BT 1        | 10,000 | 10.0 13.3 11.7 | 3.3 1.7 | 3.3 3.3 3.3 | 6.7 5.0 10.0 |
|             | 6,000  | 10.0 33.3 22.0 | 3.3 1.7 | 6.7 6.7 6.7 | 6.7 13.3 10.0 |
|             | 2,500  | 20.0 23.3 21.7 | 3.3 1.7 | 13.3 13.3 13.3 |
|             | 500    | 20.0 23.3 21.7 | 3.3 1.7 | 13.3 13.3 13.3 |
| BT 2        | 200    | 11.7 8.3 10.0 | 3.3 1.7 | 6.7 5.0 5.8 | 18.3 10.0 14.1 |
|             | 150    | 11.7 8.3 10.0 | 3.3 1.7 | 6.7 5.0 5.8 | 18.3 10.0 14.1 |
|             | 100    | 11.7 8.3 10.0 | 3.3 1.7 | 6.7 5.0 5.8 | 18.3 10.0 14.1 |
| BT 1, BT 9  | 50     | 11.7 8.3 10.0 | 3.3 1.7 | 6.7 5.0 5.8 | 18.3 10.0 14.1 |
| BT 15       | 25     | 11.7 8.3 10.0 | 3.3 1.7 | 6.7 5.0 5.8 | 18.3 10.0 14.1 |
|             | 10     | 11.7 8.3 10.0 | 3.3 1.7 | 6.7 5.0 5.8 | 18.3 10.0 14.1 |
|             | 5      | 11.7 8.3 10.0 | 3.3 1.7 | 6.7 5.0 5.8 | 18.3 10.0 14.1 |
|             | 1      | 11.7 8.3 10.0 | 3.3 1.7 | 6.7 5.0 5.8 | 18.3 10.0 14.1 |
| Control     | 0      | 11.7 8.3 10.0 | 3.3 1.7 | 6.7 5.0 5.8 | 18.3 10.0 14.1 |

Table 34. Incidence of ELAS, and ELA in Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiments | Concentration, ppm | Animals with tumors, % |
|-------------|-------------------|------------------------|
|             |                   | ELAS  | ELA  |
|             | M     F     Total | M     F     Total |
| BT 6        | 30,000 | 3.3 1.7 | 3.3 |
| BT 1        | 10,000 | 3.3 1.7 | 3.3 |
|             | 6,000  | 3.3 1.7 | 3.3 |
|             | 2,500  | 3.3 1.7 | 3.3 |
|             | 500    | 3.3 1.7 | 3.3 |
| BT 2        | 200    | 3.3 1.7 | 3.3 |
|             | 150    | 3.3 1.7 | 3.3 |
|             | 100    | 3.3 1.7 | 3.3 |
| BT 1, BT 9  | 50     | 3.3 1.7 | 3.3 |
| BT 15       | 25     | 3.3 1.7 | 3.3 |
|             | 10     | 3.3 1.7 | 3.3 |
|             | 5      | 3.3 1.7 | 3.3 |
|             | 1      | 3.3 1.7 | 3.3 |
| Controls    | 0      | 3.3 1.7 | 3.3 |

Table 35. Incidence of hepatomas, neoplastic liver nodules, nodular hyperplasia of the liver and diffuse hyperplasia of the liver in Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiments | Concentration, ppm | Animals with tumors and correlated changes, % |
|-------------|-------------------|---------------------------------------------|
|             |                   | Hepatomas  | Neopl. nod. | Nod. hyp. | Diff. hyp. |
|             | M     F     Total | M     F     Total | M     F     Total | M     F     Total |
| BT 6        | 30,000 | 3.3 1.7 | 3.3 1.7 | 3.3 1.7 | 3.3 1.7 |
| BT 1        | 10,000 | 3.3 1.7 | 3.3 1.7 | 3.3 1.7 | 3.3 1.7 |
|             | 6,000  | 3.3 1.7 | 3.3 1.7 | 3.3 1.7 | 3.3 1.7 |
|             | 500    | 3.3 1.7 | 3.3 1.7 | 3.3 1.7 | 3.3 1.7 |
|             | 250    | 3.3 1.7 | 3.3 1.7 | 3.3 1.7 | 3.3 1.7 |

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### Table 35 (cont.)

Animals with tumors and correlated changes, %

| Experiments | Concentration, ppm | Hepatomas | Neopl. nod. | Nod. hyp. | Diff. hyp. |
|-------------|-------------------|-----------|-------------|-----------|------------|
|             |                   | M F Total | M F Total   | M F Total | M F Total  |
| BT 2        | 200               | 1.7 3.3 2.5 | 3.3 1.7 2.5 | 20.0 13.3 16.7 | 38.3 18.3 28.3 |
|             | 150               | - - -     | - - -       | 8.3 13.3 10.8 | 16.7 25.3 20.8 |
|             | 100               | - - -     | - - -       | 5.0 23.3 14.2 | 26.7 13.3 20.0 |
| BT 1, BT 9  | 50                | - - 0.5   | - - 0.3     | 13.3 9.4 11.4 | 2.8 3.3 3.0  |
| BT 15       | 25                | - - -     | - - -       | 8.3 11.7 5.0 10.0 | 6.7 8.3 7.5  |
|             | 10                | - - -     | - - -       | 15.0 5.0 12.5 | 10.0 6.7 8.3  |
|             | 5                 | - - -     | - - -       | 5.0 1.7 0.8   | - - -      |
|             | 1                 | - - -     | - - -       | 3.3 1.7 1.7   | 0.9 2.9 1.9 |
| Controls    | 0                 | - - -     | - - -       | 0.4 0.2 0.4   | 0.8 0.9 2.9 |

### Table 36. Incidence of nephroblastoma in Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiment | Concentration, ppm | Animals with NEPHRO-BL, % |
|------------|--------------------|----------------------------|
|            |                    | M  | F  | Total |
| BT 1       | 30,000             | -  | -  | -     |
| BT 2       | 10,000             | 6.7| 6.7| 11.7  |
|            | 6,000              | 13.8| 3.3| 5.8       |
|            | 2,500              | 16.7| 3.3| 10.0     |
|            | 500                | -  | -  | -     |
|            | 250                | 3.4| 3.3| 6.5       |
|            | 200                | 3.3| 3.3| 6.6       |
|            | 100                | 13.3| 3.3| 8.3       |
| BT 1, BT 9 | 50                 | -  | 1.1| 1.1       |
| BT 15      | 25                 | 1.7| -  | 0.8       |
|            | 10                 | -  | -  | -     |
|            | 5                  | -  | -  | -     |
|            | 1                  | -  | -  | -     |
| Controls   | 0                  | -  | -  | -     |

### Table 37. Incidence of neuroblastoma in Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiments | Concentration, ppm | Animals with NEURO-BL, % |
|-------------|--------------------|----------------------------|
|             |                    | M  | F  | Total |
| BT 1        | 30,000             | 3.3| -  | 1.7       |
| BT 2        | 10,000             | 6.7| 16.7| 17.7     |
|             | 6,000              | 6.9| 3.3| 5.1       |
|             | 2,500              | 6.7| 6.7| 6.7       |
|             | 500                | -  | -  | -     |
|             | 250                | -  | -  | -     |
|             | 200                | -  | -  | -     |
|             | 150                | -  | -  | -     |
|             | 100                | -  | -  | -     |
| BT 1, BT 9  | 50                 | -  | -  | -     |
| BT 15       | 25                 | -  | -  | -     |
|             | 10                 | -  | -  | -     |
|             | 5                  | -  | -  | -     |
|             | 1                  | -  | -  | -     |
| Control     | 0                  | -  | -  | -     |

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Table 38. Incidence of zymbal gland carcinoma in Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiment | Concentration, ppm | M | F | Total |
|------------|-------------------|---|---|-------|
| BT 6       | 30,000            | 56.6 | 60.0 | 58.3 |
| BT 1       | 10,000            | 33.3 | 20.0 | 26.7 |
|            | 6,000             | 10.3 | 13.3 | 11.9 |
|            | 2,500             | 3.3  | 3.3  | 3.3  |
|            | 500               | 10.0 | 3.3  | 6.7  |
|            | 250               | -    | -    | -    |
| BT 2       | 200               | 5.0  | 1.7  | 3.3  |
|            | 150               | -    | 1.7  | 3.4  |
|            | 100               | -    | -    | 0.8  |
| BT 1, BT 9 | 50                | 2.3  | 2.8  | 2.5  |
| BT 15      | 25                | 5.0  | 1.7  | 3.3  |
|            | 10                | 1.7  | 1.7  | 1.7  |
|            | 5                 | -    | 1.7  | 0.8  |
|            | 1                 | -    | 0.8  | -    |
| Controls   |                   | 0.9  | 0.8  | 0.9  |

Table 39. Incidence of forestomach papilloma and acanthoma in Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiment | Concentration, ppm | M | F | Total |
|------------|-------------------|---|---|-------|
| BT 6       | 30,000            | 16.7 | 20.0 | 18.3 |
| BT 1       | 10,000            | -  | -  | -    |
|            | 6,000             | -  | 3.3 | 1.7  |
|            | 2,500             | -  | -  | -    |
|            | 500               | -  | -  | -    |
|            | 250               | -  | -  | -    |
| BT 2       | 200               | -  | -  | -    |
|            | 150               | 3.3 | -  | 1.7  |
|            | 100               | 3.3 | 3.3 | 3.3  |
| BT 1, BT 9 | 50                | 1.1 | -  | 0.6  |
| BT 15      | 25                | -  | -  | -    |
|            | 10                | -  | -  | -    |
|            | 5                 | -  | -  | -    |
|            | 1                 | -  | -  | -    |
| Controls   |                   | 1.3 | 0.4 | 0.9  |

their onset, even at doses below the ones with statistically significant results.

An excess of Zymbal gland carcinomas is observed down to 50 and 25 ppm.

Liver angiosarcomas are extremely rare in the colony used (4 cases over several thousand untreated animals). Therefore, one must consider the onset of these tumors as important even at doses not shown by statistical analysis, and particularly below 50 ppm (5 liver angiosarcomas out of 120 animals at 25 ppm, and 1 liver angiosarcoma out of 120 animals at 10 ppm), and at 1 mg/kg (3 liver angiosarcomas out of 150 animals), and at 0.3 mg/kg (1 liver angiosarcoma out of 150 animals).

The onset of a few nephroblastomas observed after inhalation treatment at doses below 100 ppm and in groups treated by ingestion with 50 and 16.65 mg/kg, is not casual in our opinion, given the extreme rarity of these tumors in rats.

Neuroblastomas have never been observed by us, up to the present, in the Sprague-Dawley rats used in our laboratory as control or otherwise.

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Table 40. Incidence of mammary malignant tumor in female Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiment | Concentration, ppm | Animals with Mammary MT, % |
|------------|--------------------|-----------------------------|
| BT 6       | 30,000             | 6.7                         |
| BT 1       | 10,000             | 10.0                        |
|           | 6,000              | -                           |
|           | 2,500              | 6.7                         |
|           | 500                | 3.3                         |
|           | 250                | 6.7                         |
| BT 2       | 200                | 8.3                         |
|           | 150                | 10.0                        |
|           | 100                | 6.7                         |
| BT 1       | 50                 | 6.7                         |
| BT 9       | 50                 | 40.7                        |
| BT 15      | 25                 | 28.3                        |
|           | 10                 | 35.0                        |
|           | 5                  | 38.3                        |
|           | 1                  | 23.3                        |
| Controls   |                    |                             |
| BT 1       | 0                  | -                           |
| BT 2       | 0                  | 2.0                         |
| BT 9       | 0                  | 18.0                        |
| BT 15      | 0                  | 10.0                        |

Table 41. Incidence of total MT and BT in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiment | Concentration, ppm | Tumors/100 Animals |
|------------|--------------------|--------------------|
|            |                    | MT     | BT     |
| BT 7       | 10,000             | 50.0   | 10.0   |
|           | 6,000              | 53.3   | 20.0   |
|           | 2,500              | 26.7   | 13.3   |
|           | 500                | 30.0   | 10.0   |
|           | 250                | 13.3   | 16.7   |
|           | 50                 | 16.7   | 6.7    |
| BT 17      | 1                  | 24.2   | 29.2   |
| Controls   |                    |        |        |
| BT 7       | 0                  | 15.0   | 15.0   |
| BT 17      | 0                  | 20.0   | 18.5   |

Table 42. Incidence of LAS, LA, A++++↑ and A+ in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiment | Concentration, ppm | LAS | LA  | A++++↑ | A+ |
|------------|--------------------|-----|-----|--------|----|
| BT 7       | 10,000             | 29.6| -   | 3.3    | -  |
|           | 6,000              | 11.5| 7.7 | -      | 3.3|
|           | 2,500              | 12.0| -   | 3.3    | -  |
|           | 500                | 10.7| 3.6 | 3.3    | -  |
|           | 250                | 3.7 | -   | 3.3    | -  |
|           | 50                 | -   | -   | -      | -  |
| BT 17      | 1                  | -   | 1.0 | 1.7    | 0.8|
| Controls   |                    | -   | -   | -      | -  |
| BT 7, BT 17|                    | -   | -   | -      | -  |

Table 43. Incidence of ELAS and ELA in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiment | Concentration, ppm | ELAS | ELA |
|------------|--------------------|------|-----|
| BT 7       | 10,000             | -    | -   |
|           | 6,000              | 3.8  | 3.8 |
|           | 2,500              | 4.0  | -   |
|           | 500                | -    | -   |
|           | 250                | 3.7  | 3.7 |
|           | 50                 | -    | -   |
| BT 17      | 1                  | 3.0  | 5.0 |
| Controls   |                    | 0.7  | -   |
| BT 7, BT 17|                    | -    | -   |

treated. Therefore we consider as dependent on treatment the onset of these tumors, even at doses below 10,000 ppm, i.e., 6000 and 2500 ppm.

The meaning in oncological terms of the results at the lowest doses may be better evaluated in considering, not singly, but together, the tumors found to be VC-dependent (Table 71).

None (or no increase) of the specifically VC related tumors shown in Table 71, observed in the seven basic experiments, was found at doses of 5 and 1 ppm (by inhalation) and 0.03 mg/kg (by ingestion).

**General Comments**

VC long-term experimental study led to the discovery of VC carcinogenicity, and as a direct consequence, to what probably has been the greatest effort ever made at controlling the exposure to an industrial carcinogen in the workplace (Table 72).

Moreover, long-term carcinogenicity bioassays on VC are a crucial step in the field of environmental and occupational carcinogenesis which, in turn,
Table 44. Incidence of hepatomas, neoplastic liver nodules, nodular hyperplasia of the liver and diffuse hyperplasia of the liver in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiment | Concentration, ppm | Hepatomas | Neop.nod. | Nod.hyp. | Diff.hyp. |
|------------|-------------------|-----------|-----------|----------|-----------|
| BT 7       | 10,000            | –         | –         | 6.7      | –         |
|            | 6,000             | –         | 7.7       | 6.7      | 3.3       |
|            | 2,500             | 4.0       | –         | 6.7      | 6.7       |
|            | 500               | –         | 6.7       | –        | 3.3       |
|            | 250               | –         | –         | –        | 16.7      |
|            | 50                | –         | –         | –        | 10.0      |
| BT 17      | 1                 | 1.0       | –         | –        | 5.0       |
| Controls   | 0                 | –         | –         | 1.2      | 2.3       |
| BT 7, BT 17|                   |           |           |          |           |

Table 45. Incidence of NEPHRO-BL in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiment | Concentration, ppm | Animals with NEPHRO-BL, % |
|------------|--------------------|---------------------------|
| BT 7       | 10,000             | 3.7                       |
|            | 6,000              | 7.7                       |
|            | 2,500              | –                         |
|            | 500                | 7.1                       |
|            | 250                | –                         |
|            | 50                 | 3.6                       |
| BT 17      | 1                  | –                         |
| Controls   | 0                  | –                         |
| BT 7, BT 17|                   |                           |

Table 46. Incidence of NEURO-BL in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiment | Concentration, ppm | Animals with NEURO-BL, % |
|------------|--------------------|--------------------------|
| BT 7       | 10,000             | 11.1                     |
|            | 6,000              | 3.8                      |
|            | 2,500              | 4.0                      |
|            | 500                | –                        |
|            | 250                | –                        |
|            | 50                 | –                        |
| BT 17      | 1                  | –                        |
| Controls   | 0                  | –                        |
| BT 7, BT 17|                   |                           |

Table 47. Incidence of Zymbalg gland CA in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiment | Concentration, ppm | Animals with Zymbalg gland CA, % |
|------------|--------------------|---------------------------------|
| BT 7       | 10,000             | 7.4                             |
|            | 6,000              | 7.7                             |
|            | 2,500              | –                               |
|            | 500                | –                               |
|            | 250                | –                               |
|            | 50                 | –                               |
| BT 17      | 1                  | 2.0                             |
| Controls   | 0                  | 2.3                             |
| BT 7, BT 17|                   |                                 |

Table 48. Incidence of forestomach Pa and Ac in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

| Experiment | Concentration, ppm | Animals with forestomach Pa and Ac, % |
|------------|--------------------|--------------------------------------|
| BT 7       | 10,000             | –                                    |
|            | 6,000              | –                                    |
|            | 2,500              | –                                    |
|            | 500                | –                                    |
|            | 250                | –                                    |
|            | 50                 | –                                    |
| BT 17      | 1                  | –                                    |
| Controls   | 0                  | 0.7                                  |
| BT 7, BT 17|                   |                                      |

are among the most important areas of public health nowadays.

These studies have demonstrated that long-term carcinogenicity bioassays: may predict carcinogenic risk for humans; may give indication of the level of risk, in relation to dose; may provide information on possible target organs and, in general terms, on the quality of neoplastic response; may represent a tool for obtaining information on the relative risk represented by different compounds, provided that they are tested under the same standard conditions (Table 73); have revealed the need to identify

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Table 49. Incidence of total MT and BT in Swiss mice in relation to concentration of VC administered by inhalation for 30 weeks.

| Experiment | Concentration, ppm | MT       | BT       |
|------------|-------------------|----------|----------|
|            |                   | M        | F        | Total    | M        | F        | Total    |
| BT 4       | 10,000            | 16.6     | 83.3     | 50.0     | 83.3     | 113.3    | 98.3     |
|            | 6,000             | 26.7     | 86.6     | 56.7     | 100.0    | 100.0    | 100.0    |
|            | 2,500             | 40.0     | 76.7     | 58.3     | 80.0     | 100.0    | 90.0     |
|            | 500               | 36.7     | 80.0     | 58.3     | 93.3     | 113.3    | 103.3    |
|            | 250               | 36.7     | 90.0     | 63.3     | 116.7    | 80.0     | 98.3     |
|            | 50                | 6.2      | 50.0     | 28.3     | 20.0     | 26.7     | 23.3     |
|            | 0                 |          |          |          |          |          |          |

Table 50. Incidence of LAS and LA in Swiss mice in relation to concentration of VC administered by inhalation for 30 weeks.

| Experiment | Concentration, ppm | LAS   | LA   |
|------------|-------------------|------|------|
|            |                   | M    | F    | Total | M    | F    | Total |
| BT 4       | 10,000            | 3.8  | 30.0 | 17.8  | 3.8  | 16.7 | 10.7  |
|            | 6,000             | 6.7  | 36.7 | 21.7  | 6.7  | 16.7 | 11.7  |
|            | 2,500             | 20.7 | 33.3 | 27.1  | 6.9  | 10.0 | 8.5   |
|            | 500               | 20.0 | 26.7 | 23.3  | 3.3  | 13.3 | 8.3   |
|            | 250               | 30.0 | 30.0 | 30.0  | 20.0 | 16.7 | 18.3  |
|            | 50                | 3.3  | -    | 1.7   | -    | 3.3  | 1.7   |
|            | 0                 | -    | -    | -     | -    | -    | -     |

Table 51. Incidence of ELAS and ELA in Swiss mice in relation to concentration of VC administered by inhalation for 30 weeks.

| Experiment | Concentration, ppm | ELAS   | ELA   |
|------------|-------------------|--------|-------|
|            |                   | M      | F     | Total | M    | F     | Total |
| BT 4       | 10,000            | -      | 3.3   | 1.8   | 7.7  | 6.7   | 7.1   |
|            | 6,000             | -      | 3.3   | 1.7   | 6.7  | 3.3   | 5.0   |
|            | 2,500             | 13.8   | 13.3  | 13.5  | 6.7  | 3.3   | 5.0   |
|            | 500               | 6.7    | 16.7  | 11.7  | 3.3  | 6.7   | 5.0   |
|            | 250               | 6.7    | 3.3   | 5.0   | 6.7  | 3.3   | 5.0   |
|            | 50                | 3.3    | -     | 1.7   | 3.3  | 13.3  | 8.3   |
|            | 0                 | -      | 1.4   | 0.7   | 1.2  | -     | 0.7   |

Table 52. Incidence of lung tumors (Ad and Ad ↑) in Swiss mice in relation to concentration of VC administered by inhalation for 30 weeks.

| Experiment | Concentration, ppm | Animals with lung tumors (Ad and Ad ↑), % |
|------------|-------------------|------------------------------------------|
|            |                   | M    | F    | Total |
| BT 4       | 10,000            | 76.9 | 86.7 | 82.1  |
|            | 6,000             | 76.7 | 80.0 | 78.3  |
|            | 2,500             | 62.1 | 73.3 | 67.8  |
|            | 500               | 80.0 | 86.7 | 83.3  |
|            | 250               | 80.0 | 56.7 | 68.3  |
|            | 50                | 10.0 | 10.0 | 10.0  |
|            | 0                 | 10.0 | 10.0 | 10.0  |
Table 53. Incidence of mammary CA in female Swiss mice in relation to concentration of VC administered by inhalation for 30 weeks.

| Experiment | Concentration, ppm | Animals with mammary CA, % |
|------------|--------------------|-----------------------------|
| BT 4       | 10,000             | 43.3                        |
|            | 6,000              | 26.7                        |
|            | 2,500              | 26.7                        |
|            | 500                | 23.3                        |
|            | 250                | 40.0                        |
|            | 50                 | 40.0                        |
|            | 0                  | 1.4                         |

animal systems more equivalent to humans in neoplastic response, which in turn depends on partly-known factors, such as basic “spontaneous” tumorigram and enzymatic profiles.

**Prospects**

At present the most important goal of research on environmental and occupational carcinogenesis is, in our own view, the extrapolation of results

Table 54. Incidence of forestomach Pa and Ca in Swiss mice in relation to concentration of VC administered by inhalation for 30 weeks.

| Experiment | Concentration, ppm | M | F | Total |
|------------|--------------------|---|---|-------|
| BT 4       | 10,000             | 3.3| 1.8| 5.1  |
|            | 6,000              | 3.3| -  | 3.3  |
|            | 2,500              | 3.3| -  | 3.3  |
|            | 500                | 3.3| -  | 3.3  |
|            | 250                | 3.3| -  | 3.3  |
|            | 50                 | 3.3| -  | 3.3  |
|            | 0                  | -  | -  | -    |

Table 55. Incidence of total MT and BT in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

| Experiment | Concentration, mg/kg | MT | BT |
|------------|----------------------|----|----|
|            |                      | M  | F  | Total | M  | F  | Total |
| BT 11      | 50.00                | 35.0| 15.0| 50.0| 35.0| 15.0| 50.0|
|            | 16.65                | 22.5| 14.7| 37.2| 20.0| 30.0| 50.0|
|            | 3.33                 | 5.0 | 15.0| 20.0| 2.5 | 47.5| 50.0|
| BT 27      | 1.0                  | 13.3| 24.7| 38.0| 12.0| 58.7| 70.7|
|            | 0.3                  | 12.0| 28.0| 40.0| 20.0| 36.0| 56.0|
|            | 0.03                 | 8.0 | 15.0| 23.0| 14.7| 48.0| 62.7|
| Controls   | BT 11                | 0   | 12.5| 12.5| 10.0| 35.0| 45.0|
|            | BT 27                | 0   | 8.0 | 8.0 | 9.3 | 48.0| 57.3|

Table 56. Incidence of LAS, LA, A + + + + and A + / + + + in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

| Experiment | Concentration, mg/kg | LAS | LA | A + + + + | A + / + + + |
|------------|----------------------|-----|----|----------|------------|
|            |                      | M   | F  | Total    | M   | F  | Total |
| BT 11      | 50.00                | 20.0| 22.5| 22.5    | 2.5 | 5.0| 7.5  |
|            | 16.65                | 10.0| 15.0| 12.5    | 2.5 | 5.0| 7.5  |
|            | 3.33                 | -   | -   | -       | 2.5 | 7.5| 10.0 |
| BT 27      | 1.0                  | 1.3 | 2.7 | 2.0     | -   | -  | -    |
|            | 0.3                  | -   | 1.4 | 0.7     | -   | 1.4| 1.3  |
|            | 0.03                 | -   | -   | -       | -   | -  | -    |
| Controls   | BT 11, BT 27         | 0   | -   | -       | 0.9 | 0.4| 0.9  |

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Table 57. Incidence of ELAS and ELA in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

| Experiment | Concentration, mg/kg | M  | F  | Total | ELAS | M  | F  | Total |
|------------|----------------------|----|----|-------|------|----|----|-------|
| BT 11      | 50.00                | 5.0|    | 2.5   | 2.5  |    |    | 2.5   |
|            | 16.65                |    |    |       |      |    |    |       |
|            | 3.33                 |    |    |       |      |    |    |       |
| BT 27      | 1.0                  |    | 1.3| 0.7   | -    |    |    |       |
|            | 0.3                  |    |    |       |      |    |    |       |
|            | 0.03                 |    |    |       |      |    |    |       |
| Controls   | BT 11, BT 27         |    |    |       |      |    |    |       |

Animals with tumors, %

Table 58. Incidence of hepatomas, neoplastic liver nodules, nodular hyperplasia of the liver, and diffuse hyperplasia of the liver in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

| Experiment | Concentration, mg/kg | M  | F  | Total | Hepatomas | M  | F  | Total | Neop.nod. | M  | F  | Total | Nod.hyp. | M  | F  | Total | Dif.hyp. | M  | F  | Total |
|------------|----------------------|----|----|-------|-----------|----|----|-------|-----------|----|----|-------|----------|----|----|-------|----------|----|----|-------|
| BT 11      | 50.00                |    |    |       | 5.0       |    |    |       | 1.3       |    |    |       | 2.7       |    |    |       | 2.0       |    |    |       |
|            | 16.65                |    |    |       | 2.5       |    |    |       | 1.3       |    |    |       | 2.7       |    |    |       | 1.2       |    |    |       |
|            | 3.33                 |    |    |       |           |    |    |       |           |    |    |       |           |    |    |       |           |    |    |       |
| BT 27      | 1.0                  | 0.7| 0.7| 0.7   | 1.3       |    |    |       | 1.3       |    |    |       | 0.7       |    |    |       | 0.7       |    |    |       |
|            | 0.3                  |    |    |       |           |    |    |       |           |    |    |       |           |    |    |       |           |    |    |       |
|            | 0.03                 |    |    |       |           |    |    |       |           |    |    |       |           |    |    |       |           |    |    |       |
| Controls   | BT 11, BT 27         |    |    |       |           |    |    |       |           |    |    |       |           |    |    |       |           |    |    |       |

Animals with tumors and correlated changes, %

Table 59. Incidence of NEPHRO-BL in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

| Experiment | Concentration, mg/kg | M  | F  | Total | NEPHRO-BL, % |
|------------|----------------------|----|----|-------|--------------|
| BT 11      | 50.00                | 2.5| 2.5| 2.5   | 2.5          |
|            | 16.65                | 5.0| 2.5| 3.7   |              |
|            | 3.33                 |    |    |       |              |
| BT 27      | 1.0                  |    |    |       |              |
|            | 0.3                  |    |    |       |              |
|            | 0.03                 |    |    |       |              |
| Controls   | BT 11, BT 27         |    |    |       |              |

Table 60. Incidence of NEURO-BL in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

| Experiment | Concentration, mg/kg | M  | F  | Total |
|------------|----------------------|----|----|-------|
| BT 11      | 50.00                | 2.5| 2.5| 2.5   |
|            | 16.65                | 3.3| 3.3| 3.3   |
|            | 3.33                 | 1.0| 1.0| 1.0   |
| BT 27      | 0.3                  |    |    |       |
|            | 0.03                 |    |    |       |
| Controls   | BT 11, BT 27         |    |    |       |

from animal to human, both in qualitative and in quantitative terms.

VC carcinogenicity may again provide an important tool towards solving this problem.

We now know a great deal about the effects of VC in experimental animal systems, both in qualitative and quantitative terms.

On the other hand, epidemiological investigations on occupationally exposed population groups have been made and are being carried out in different parts of the world, particularly in Western Europe and in the U.S.A., with reference to general pathology and neoplasias.

If these epidemiological investigations provide precise figures on the whole group considered, including figures on the level and length of expo-
Table 61. Incidence of Zymbal gland CA in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

| Experiments | Concentration, mg/kg | Animals with Zymbal gland CA, % |
|-------------|----------------------|---------------------------------|
|             |                      | M | F | Total |
| BT 11       | 50.00                | 2.5 | - | 1.2   |
|             | 16.65                | 2.5 | 2.5 | 2.5   |
|             | 3.33                 |    | - | -     |
| BT 27       | 1.0                  | 2.7 | 4.0 | 3.3   |
|             | 0.3                  |    | - | -     |
|             | 0.03                 |    | - | -     |
| Controls    | 0                    |    | - | 1.7   |

Table 62. Incidence of forestomach Pa and Ac in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

| Experiments | Concentration, mg/kg | Animals with forestomach Pa and Ac, % |
|-------------|----------------------|---------------------------------------|
|             |                      | M | F | Total |
| BT 11       | 50.00                | 5.0 | - | 2.5   |
|             | 16.65                |    | 2.5 | 1.2   |
|             | 3.33                 |    | - | -     |
| BT 27       | 1.0                  | 1.3 | 2.7 | 2.0   |
|             | 0.3                  |    | - | -     |
|             | 0.03                 |    | - | 1.3   |
| Controls    | 0                    |    | - | 1.7   |

Table 63. Incidence of mammary MT in female Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

| Experiment | Concentration, mg/kg | Animals with mammary MT, % |
|------------|----------------------|-----------------------------|
| BT 11      | 50.00                | 10.0                        |
|            | 16.65                | 15.0                        |
|            | 3.33                 | 5.0                         |
| BT 27      | 1.0                  | 16.0                        |
|            | 0.3                  | 5.5                         |
|            | 0.03                 | 18.7                        |
| Controls   | BT 11                | 0                           |
|            | BT 27                | 9.3                         |

sure (so as to define homogeneous exposed groups), and collect all possible available data on pathology, we shall have an opportunity, unique at present, to compare animal and human data, both in qualitative and quantitative terms, and to help find a possible key for extrapolating from animals to humans.

The Cost

With the presentation made in Paris last November (2) and with today's report, ten years of work on our VC experimental project seem to be nearly concluded. After having presented the results, we also wish to present the data of the cost of the project, which cannot be expressed only in financial terms.

The cost of the BT project of long-term carcinogenicity bioassays on vinyl chloride includes the cost of (1) the planning and setting-up of experimental apparatus, including inhalation facilities, of

Table 64. Incidence of LAS and Zymbal gland CA in Sprague-Dawley rats in relation to schedule of treatment with VC administered by inhalation.

| Experiment | VC concentration, ppm | Schedulea | Animals with tumors, % |
|------------|------------------------|-----------|------------------------|
|            |                        |           | LAS | Total | Zymbal gland CA | Total |
|            |                        |           | M | F | Total | M | F | Total |
| BT 1       | 10,000                 | I         | 10.0 | 13.3 | 11.7 | 33.3 | 20.0 | 26.7 |
| BT 3       | 10,000                 | II        | - | - | - | 17.8 | 13.3 | 15.5 |
| BT 10      | 10,000                 | III       | 1.7 | - | 0.8 | 13.5 | 1.7 | 7.6 |
|            |                        | IV        | 1.7 | - | 0.8 | 8.5 | 6.7 | 7.6 |
| BT 1       | 6,000                  | V         | - | 1.7 | 0.8 | 3.3 | 10.2 | 6.7 |
| BT 3       | 6,000                  | I         | 10.3 | 33.3 | 22.0 | 10.3 | 12.3 | 11.9 |
| BT 10      | 6,000                  | II        | - | 3.3 | 1.7 | 20.0 | 10.0 | 15.0 |
|            |                        | III       | - | - | - | 10.0 | 5.0 | 7.5 |
|            |                        | IV        | 3.4 | 1.7 | 2.5 | 8.5 | - | 4.2 |
|            |                        | V         | - | 1.7 | 0.8 | 10.0 | 5.0 | 7.5 |

*aSchedules: (I) 4 hr/day, 5 days/wk, 52 weeks; (II) 4 hr/day, 5 days/wk, 17 weeks; (III) 4 hr/day, 5 days/wk, 5 weeks; (IV) 1 hr/day, 4 days/wk, 25 weeks; (V) 4 hr/day, 1 day/wk, 25 weeks.
Table 65. Incidence of LAS in relation to species (male Sprague-Dawley rats, Wistar rats, Swiss mice and golden hamsters), treated with VC administered by inhalation.

| Experiments | Concentration, ppm | Sprague-Dawley rats | Wistar rats | Swiss mice | Golden hamsters |
|-------------|--------------------|---------------------|-------------|------------|----------------|
| BT1, BT7, BT4, BT8 | 10,000 | 10.0 | 29.6 | 3.8 | – |
|              | 6,000  | 10.3 | 11.5 | 6.7 | 3.3 |
|              | 2,500 | 20.0 | 12.0 | 20.7 | – |
|              | 500   | –    | 10.0 | 20.0 | – |
|              | 250   | 3.4  | 3.7  | 30.0 | – |
|              | 50    | –    | –    | –    | 3.3 |
|              | 0     | –    | –    | –    | – |

Table 66. Incidence of Zymbal gland CA in relation to strain (male Sprague-Dawley and Wistar rats) treated with VC administered by inhalation.

| Experiments | Concentration, ppm | Sprague-Dawley rats | Wistar rats |
|-------------|--------------------|---------------------|-------------|
| BT1, BT7    | 10,000             | 33.3                | 7.4         |
|             | 6,000              | 10.3                | 7.7         |
|             | 2,500              | 3.3                 | –           |
|             | 500                | 10.0                | –           |
|             | 250                | –                   | –           |
|             | 50                 | –                   | –           |
|             | 0                  | –                   | –           |

Table 67. Incidence of LAS in relation to age (newborn and adult) Sprague-Dawley rats treated with VC administered by inhalation 4 hr/day, 5 days/week, 52 weeks.

| Experiment | Concentration, ppm | Newborn rats | 11 week old rats |
|------------|--------------------|--------------|------------------|
|            |                    | M | F | Total | M | F | Total |
| BT 10, BT 14 | 10,000            | 25.0 | 45.0 | 34.1 | 1.7 | – | 0.8 |
|            | 6,000              | 27.8 | 50.0 | 40.5 | – | – | – |

Table 68. Tumors presently correlated to VC exposure, by experiments on rodents.

| Species | Angiosarcomas of liver | Tumors of brain | Tumors of lung | Lymphomas and leukemias | Hepatomas | Angiosarcomas and angiomatous other sites | Nephroblastomas | Sebaceous cutaneous carcinomas | Other cutaneous epithelial tumours | Mammary carcinomas | Forestermack papillomas and acanthomas | Melanomas |
|---------|------------------------|-----------------|---------------|--------------------------|-----------|------------------------------------------|----------------|-----------------------------|-------------------------------|------------------|--------------------------------------|----------|
| Rat     | +                      | +               |               |                          |           |                                         |                |                             |                               |                  |                                      |          |
| Mouse   | +                      | +               |               |                          |           |                                         |                |                             |                               |                  |                                      |          |
| Hamster | +                      |                 | (+)           |                          |           |                                         |                |                             |                               |                  |                                      | (+)      |

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Table 69. Total cancer-bearing animals significantly in excess by Fisher exact probability test ($p \leq 0.05$).

| Sex | Dose level at which total cancer bearing animals in excess |
|-----|----------------------------------------------------------|
| Male | 30,000 |
|     | 10,000 |
|     | 6,000  |
|     | 2,500  |
|     | 500    |
|     | 250    |
|     | 200    |
|     | 50 ppm |
|     | 50 mg/kg |
| Female | 30,000 |
|        | 10,000 |
|        | 6,000  |
|        | 2,500  |
|        | 500    |
|        | 200    |
|        | 150    |
|        | 50 ppm |
|        | 50 mg/kg |

Table 70. Tumors significantly in excess by Fisher exact probability test ($p \leq 0.05$).

| Tumor type                  | Sex | Doses at which tumors |
|-----------------------------|-----|-----------------------|
| Zymbal gland carcinoma      | M   | 30,000; 10,000 ppm    |
|                             | F   | 30,000; 10,000 ppm    |
| Liver angiosarcoma          | M   | 30,000; 2500; 200 ppm |
|                             | F   | 30,000; 6000; 2500; 500 |
| Nephroblastoma              | M   | 2500; 200; 150; 100 ppm |
| Neuroblastoma               | F   | 10,000 ppm            |
| Mammary gland adenocarcinoma | F | 150; 50; 25; 10; 5 ppm |
| Forestromach papilloma      | M   | 30,000 ppm            |
|                             | F   | 30,000 ppm            |

Table 71. Onset of tumors considered VC-correlated at the lowest doses.

| Dose | Tumors                                                                 |
|------|------------------------------------------------------------------------|
| 25 ppm | Over 120 animals, 5 liver angiosarcomas, 4 Zymbal gland carcinomas, and 1 nephroblastoma |
| 10 ppm | Over 120 animals, 1 liver angiosarcoma, 2 extrathoracic angiosarcomas, and 2 Zymbal gland carcinomas |
| 1 mg/kg | Over 150 animals, 3 liver angiosarcomas, 1 extrathoracic angiosarcoma, 1 hepatoma, and 5 Zymbal gland carcinomas |
| 0.3 mg/kg | Over 150 animals, 1 liver angiosarcoma and 1 hepatoma |

Table 72. History of vinyl chloride carcinogenicity studies.

| Date      | VC was found to produce liver enlargement and microscopic hepatic degenerative changes (5) |
|-----------|------------------------------------------------------------------------------------------|
| 1970      | Zymbal gland carcinomas were reported in rats exposed to 30,000 ppm of VC, by inhalation (6) |
| 1970      | An increase in atypias in respiratory cells was observed among workers heavily exposed to VC (7) |
| July 1971 | A vast project of long-term carcinogenicity bioassays on VC was started in Bentivoglio, near Bologna, Italy (BT project) |
| August 1972 | Zymbal gland carcinomas, nephroblastomas and liver angiosarcomas were observed in rats exposed to VC by inhalation (Maltoni, BT project) |
| April 1973 | The first data of the BT project were released to the scientific community: the oncogenic effect was observed up to 250 ppm (4) |
| 1973      | Splenomegalic liver disease was found among poly(vinyl chloride) production workers (8) |
| December 1973 | For the first time a case of liver angiosarcoma in a poly(vinyl chloride) production worker was correlated to VC exposure (9) |
| February 1974 | On the basis of the BT project data indicating a carcinogenic effect at 250 ppm, OSHA proposed a TLV of 50 ppm |
| February 1974 | The BT project data showed that VC is a multipotential carcinogen, producing a variety of tumors, in different animal species |
| 1974      | The BT project data indicated a carcinogenic effect at 50 ppm (10); OSHA proposed new stricter rules |
| 1974      | Early epidemiological observations (paralleling the experimental information) indicated an increase in tumors other than liver angiosarcomas (of brain, lung, liver, hemolymphoreticular tissues) among workers of VC-PVC industries (11) |
| 1974-75   | BT project data showed that VC had carcinogenic effects in rats also when given by ingestion (12) |
| 1976      | In rats of the BT project exposed to VC by inhalation, angiosarcomas were observed down to the level of 25 ppm, and Zymbal gland carcinomas down to the level of 10 ppm (13) |

consistency of the methodology, which has as its reverse side the limits placed on the exercise of imagination—the most positive element in scientific life; (9) the effort involved in establishing and preserving objectivity and balance in the evaluation and interpretation of data; (10) and finally, the strength required to withstand the sense of loneliness arising from the lack of co-operation of many of those bodies which should properly be concerned with the progress of science in this field, not excluding part of the scientific community whose

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Table 73. Comparative effects of three related compounds—vinyl chloride (VC), vinylidene chloride (VDC) and ethylene dichloride (EDC) on the same animal systems.

| Compound | Species          | Angiosarcomas of liver | Tumors of the brain | Tumors of the lung | Hepatomas | Angiosarcomas and angiom as of other sites | Tumors of the kidney | Sebaceous cutaneous carcinomas | Other cutaneous epithelial tumors | Mammary carcinomas | Fore-stomach papillomas and acanthomas |
|----------|------------------|------------------------|---------------------|-------------------|-----------|-------------------------------------------|----------------------|-------------------------------|---------------------------------|-------------------|----------------------------------------|
| VC       | Rat (Sprague-Dawley) | +                      | +                   | +                 | +         | +                                        | (+)                  | +                             | (+)                             | +                 | +                                      |
|          | Mouse (Swiss)     | +                      | +                   | +                 | +         | +                                        | (+)                  | +                             | +                               | +                 | +                                      |
| VDC      | Rat (Sprague-Dawley) |                       |                     |                   |           |                                           |                      |                               |                                 | +                 | +                                      |
|          | Mouse (Swiss)     |                        |                     |                   |           |                                           |                      |                               |                                 | +                 | +                                      |
| EDC      | Rat (Sprague-Dawley) |                        |                     |                   |           |                                           |                      |                               |                                 | +                 | +                                      |
|          | Mouse (Swiss)     | +                      |                     |                   |           |                                           |                      |                               |                                 | +                 | +                                      |

indifference sometimes degenerates into frank hostility.

The high costs probably represent the reason why, in the field of experimental and environmental carcinogenesis, words overlap facts, opinions overlap data, and meetings and commissions reports submerge good laboratory work.

REFERENCES

1. Maltoni, C., Carcinogenicity of vinyl chloride: current results. Experimental evidence. (6th International Symposium on the Biological Characterization of Human Tumours, Copenhagen 1975). In: Advances in Tumour Prevention, Detection and Characterization, Excerpta Medica, Amsterdam, 1978, Vol. 3, pp. 216-227.
2. Maltoni, C., Lefemine, G., Ciliberti, A., Cotti, G., and Carretti, D. Vinyl chloride carcinogenicity bioassays (BT project) as an experimental model for risk identification and assessment in environmental and occupational carcinogenesis. In: Epidémiologie animale et épideлимologie humaine: le cas du chlorure de vinyle monomère. Publications Essentielles, Paris, 1980, pp. 103-112.
3. Maltoni, C., Lefemine, G., Ciliberti, A., Cotti, G., and Carretti, D. Vinyl chloride carcinogenicity bioassays (BT project) as an experimental model for risk identification and assessment in environmental and occupational carcinogenesis. Ospedali Vita. Field Research, Rept. 10, 7: 1-208 (1980).
4. Maltoni, C., Lefemine, G., Chieco P., and Carretti, D. La cancerogenesi ambientale e professionale: nuove prospettive alla luce della cancerogenesi da cloruro di vinile. Ospedali Vita 1 (5-6): 4-66 (1974).
5. Torkelson, T. R., Oyen, F., and Rowe, V. K. The toxicity of vinyl chloride as determined by repeated exposure of laboratory animals. Am. Ind. Hyg. Assoc. J., 22: 354 (1961).
6. Viola, P. L., Bigotti, A., and Caputo, A. Oncogenic response of rat skin, lungs and bones to vinyl chloride. Cancer Res. 31: 616-619 (1971).
7. Maltoni, C. Occupational carcinogenesis. (2nd International Symposium on Cancer Detection and Prevention, Bologna 1974) In: Advances in Tumour Prevention, Detection and Characterization, Excerpta Medica, Amsterdam, Vol. 2, 1977, p. 26.
8. Marstiller, H. J., Leibach, W. K.; Müller, R., Jübe, S., Lange, C. E., Rohner, H. G., and Veltman, G. Chronic toxic liver damage in workers of PVC producing plants. Deut. Med. Wochschr, 98: 2311-2314 (1973).
9. Creech, J. L., and Johnson, M. N. Angiosarcoma of liver in the manufacture of polyvinyl chloride. J. Occup. Med. 16: 150-151 (1974).
10. Maltoni, C., and Lefemine, G. Carcinogenicity bioassays of vinyl chloride: current results. In: Toxicity of Vinyl Chloride-Polyvinyl Chloride. New York Academy of Sciences, New York, 1975, pp. 196-218.
11. Wagoner, J. K. Statement before the Subcommittee on the Environment of the U.S. Senate Commerce Comittee, (1974).
12. Maltoni C., Ciliberti, A., Gianni, L., and Chieco, P. Insorgenza di angiosarcomi in ratti in seguito a somministrazione per via orale di cloruro di vinile. Ospedali Vita 2 (1): 65-66 (1975).
13. Maltoni C. Vinyl chloride carcinogenicity: an experimental model for carcinogenesis studies. In: Origins of Human Cancer, Cold Spring Harbor Laboratory, 1977, pp. 119-146.

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