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OCCUPATIONAL CHEMICAL CARCINOGENESIS : NEW FACTS, PRIORITIES AND PERSPECTIVES

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The first historical observation of an occupational cancer dates back to the sixteenth century: it was the so-called "mountain disease" in miners of Joachimsthal, which, only three hundred years later was known as pulmonary carcinoma, and which, only in the thirties, was recognized as due to radioactive pollution present in those mines.

Since then, up to a few years ago, the policy has been often the same: that is "let us wait and see" if an agent present in the work-place is carcinogenic or not. Which means, if it produces cancer on man, on the basis of an epidemiological evidence, of course validated by a statistical analysis.

This is not any more the time of the policy of "let us wait and see". It must be rather the starting of a new, more correct, social and scientific approach.

The reasons why occupational oncogenesis, which nowadays is mainly industrial oncogenesis, need a new approach which cannot anymore be delayed, are the following.

First. It has been estimated that from 80% to 90% of the tumours in human beings depends on causes present in the occupational and general environment, and that, therefore, cancer must be largely considered an ecological disease.

Second. The oncogenic risk has been progressively increasing in the last century, in relation to situations depending on industrial trends (table 1). Nowadays the major problem is represented by the multitude of the products of synthetic industry, which are unknown to animal and human protoplasm, and on whose effects we are therefore fully ignorant.

Third. Factories, that is the work-places, represent in the present situation the crucial area: in fact, the most exposed population lives there, and from there the new chemical compounds are spread in the general environment as consumers goods and pollution.

Fourth. There are at present clear-cut facts and knowledge on general oncogenesis whose significance is self-explanatory (table 2).

On the basis of these facts, it emerges that the only real measure to prevent industrial oncogenesis is to identify the potentially oncogenic agents, before workers and the whole mankind are exposed to them. In other words the time has come to predict the oncogenic risk, so preventing the exposure of human beings to it.

Are there nowadays available tools for this purpose? We believe so.

It is a fact that the four most important cases of environmental and occupational tumours, discovered in the last years, have been indirectly or directly predicted in some way, experimentally.

The first is the case of the clear-cell-adenocarcinoma of the vagina in adolescence, found in girls born from mothers treated during pregnancy with synthetic nonsteroid estrogen therapy. If we go back, in 1938, Lacassagne (1) already reported that he had produced mammary carcinomas in mice treated with stilbestrol and in the following years it was shown, by several scientists, that the same hormone was producing a variety of tumours in hormone dependent and non-dependent tissues, among different experimental animal species (2).

The second is the case of pulmonary carcinomas among workers exposed to bis(chloromethyl)ether. The discovery of this new type of occupational tumour came together with experimental evidence produced by Van Duuren, in 1968 (3), and by Laskin, in 1971 (4), showing respectively that, when injected subcutaneously into rats, or applied to the skin of mice, the compound was producing subcutaneous fibrosarcomas and skin carcinomas, and when inhaled by rats it induced squamous cell carcinomas of the lung.

The third and most evident example is the history of vinyl chloride carcinogenicity (5, 6, 7, 8, 9, 10).

Experimental bio-assays on the carcinogenic potential of this compound have provided information on:

- its oncogenic effects,
- the target organs and the type of tumours,
- the still effective dose levels.

Following our early observation on the dysplastic changes produced by vinyl chloride on the cells of the respiratory tract in exposed workers, and the early results of Viola (11, 12) that rats exposed to

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splastic changes produced atory tract in exposed 12) that rats exposed to

30,000 ppm of vinyl chloride developed carcinomas of the Zymbal seba- ceous gland of the external ear duct, we started in 1971 a project of integrated experiments to study the biological effects of vinyl chlori- de, in order to predict its cancerogenic risk.

A series of 17 experiments have been started in sequence.

The data of the crucial experiments are now being presented in the tables 3-5.

When administered by inhalation, vinyl chloride produced on rats Zymbal gland carcinomas, nephroblastomas, angiosarcomas of liver and of other sites, subcutaneous angiomas, skin carcinomas, hepatomas, brain neuroblastomas and mammary carcinomas. The compound shows car- cinogenic effects down to the dose level of 50 ppm.

In mice vinyl chloride produces pulmonary tumours, mammary carcino- mas, liver angiosarcomas, other vascular tumours of different sites and types, and epithelial tumours of the skin, being effective in the species also at the level of 50 ppm.

In hamsters the monomer induces liver angiosarcomas, melanomas, forestomach papillomas, acanthomas and trichoepitheliomas, and it seems to anticipate the onset of lymphomas, being the latency time 48 weeks in the treated animals and 82 weeks in controls.

Vinyl chloride is active also when given by oral administration, producing in rats some of the tumours observed following inhalatory exposure (table 6).

Moreover tumours correlated to vinyl chloride exposure have been observed on offsprings born from mothers exposed during pregnancy to high dose levels of the monomer in the air (table 7).

Before the end of 1972, a short time after it was known that vinyl chloride was inducing in rats not only Zymbal gland tumours, but also nephroblastomas and liver angiosarcomas, these results were also made known to several interested industries, and then were communicated at an international meeting in April 1973. This information promoted the clinical observation which in December 1973, for the first time, identified a liver angiosarcoma in a worker of a U.S.A. factory pro- ducing VC-PVC as occupational in origin. Since then epidemiological investigations have led to the discovery of nearly 45 liver angiosar- comas among workers of VC-PVC industries in the U.S.A. and several European countries. Most of these tumours arose before 1973 (the first known case dates from 1961), but in absence of experimental data, they were not linked to the occupational exposure, and as a matter of fact, they were often not properly diagnosed.

Vinyl chloride has caused in one or more of the three animal species used in our experiments, all types of tumours, which at present, on the basis of available epidemiological evidence, are correlated to occupational exposure, i.e. liver angiosarcomas, brain and lung tumours, lymphomas, leukemias and hepatomas (table 8).

There are now going on, in our laboratories, several experiments to better assess the degree of risk from exposure to vinyl chloride in relation to doses and length of treatment.

We are studying the effect of a short exposure (100 hours variously distributed in time) to high doses of VC on rats (table 9).

The potential oncogenic effects of exposure to 50 ppm of the monomer by inhalation is now further investigated (table 10).

A further experiment studies the effects of vinyl chloride by inhalation at doses below 50 ppm, that is 25, 10, 5 and 1 ppm (tables 11 and 12).

Finally, a few months ago, an experiment started to determine the effects of low doses of vinyl chloride by oral administration (table 13).

The fourth example of the predictive value of experimental bioassays is represented by the carcinogenicity of chromium pigments.

In 1969 we started a program of investigation on the cancerogenic risk from exposure to several inorganic compounds and pigments. A series of several of the compounds under studies, with the available results, is given in the following table (table 14).

The results dealing with lead-chromate pigments and lead-molybdenum chromate pigments were made known in April 1973 (5-13).

Data published a few months ago (14) and on going epidemiological investigations indicate an excessive incidence of bronchial carcinomas among workers producing chromium pigments.

On the basis of the present evidence it may be concluded that carcinogenicity bio-assays, if properly done, have a high predictive value.

Therefore, they cannot be any more delayed.

Up to the recent past too little importance has been given to predictive bio-assays, for many reasons, among which there were:

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nce has been given to pre- , which there were:

- ;) scepticism on their validity, based upon past results;
- 2) fatalistic acceptance of the situation because of the huge number of newly produced compounds;
- ;) complication of elaborate testings.

As regards scepticism, we think that time has come to critically review methods and results of past experiments which, nowadays, may be thought to have been inadequate.

As regards the large number of compounds, it may be true that there are already millions of newly produced chemicals, but the ones which urgently need to be tested, mainly because of their widespread diffusion, actually number in the hundreds.

Concerning complications in performing elaborate testings, it may be true that the more experimental tests on animals can reproduce the conditions of human exposure, the more relevant they are in man. It should be pointed out, however, that agents to be examined should follow a pattern of tests which progress in degree of precision, so as to filter out and expose the most dangerous.

In our opinion the future policy for the prevention of the occupational as well of the environmental oncogenesis, are the following:

- 1) priority to experimental bio-assays for the scrutiny of compounds already produced and widespread and for the compounds to be still produced on a large scale;
- 2) basic studies to investigate the potentiality of experimental models in determining the oncogenic risk with particular emphasis on easy and rapid tests which, however, should be carefully and critically evaluated; and
- 3) the study with proper long term bio-assays already validated, of the most important and widespread compounds, whose effects are still unknown, following a list of priorities determined by all the interested social parties.

On this line in our Institute we are now studying several compounds, among which styrene, acrylonitrile and vinylidene chloride. The plans of these experiments are given in the tables 15, 16, 17.

Ongoing experiments performed in our Institute on the carcinogenic potential of drugs have shown in last weeks that a drug widely used in tumour chemotherapy and also in the prophylaxis of neoplastic recurrence, namely adriamycin, is a potent carcinogen (table 18).

Concluding, occupational oncogenesis should not any more be considered a limited medical problem, but a wider scientific and social one, for whose control we look forward to the collaboration of all the interested parties of society: scientific community, unions, industries and governments.

SUMMARY

There is need for a new active approach to the problem of occupational carcinogenesis. It is no longer acceptable to adopt a "wait and see" attitude since:

1. evidence suggests that cancer is an "ecological" disease;
2. the carcinogenic risk has been increasing as industrialisation increased;
3. factory workers are the most exposed population and hazards spread from the work-place to the general population as consumer goods and pollution. It is necessary, and possible, to identify potential carcinogens before workers and the general population are exposed.

That predictive experiments could be done, is exemplified by the history of stilboestrol, bis(chloromethyl)ether, vinyl chloride and chromium pigments, all of which were reported carcinogenic in animal tests before any epidemiological results were available. Unfortunately, these results were received with scepticism because the test methods were thought to be inadequate.

It is necessary to develop the future policy of prevention by:

1. establishing a priority for testing compounds already produced and widespread in occurrence and those about to be produced on a large scale;
2. establishing experimental models for determining carcinogenic risk, especially rapid screening tests;
3. study of the more important compounds by long-term bioassays.

Full details are given of the testing of vinyl chloride monomer and preliminary results are tabulated for tests on styrene, acrylonitrile and vinylidene chloride.

RESUME

Une nouvelle approche active du problème de la cancérogenèse professionnelle est nécessaire. Adopter une attitude d'expectative n'est plus acceptable pour les raisons suivantes:

1. des risques conduisent à penser que le cancer est une maladie "écologique";
2. le risque cancérogène n'a cessé de croître avec l'industrialisation;
3. les travailleurs des usines sont la population la plus exposée et les risques se propagent du lieu de travail à la population générale comme les biens de consommation et la pollution. Il est nécessaire, et possible, d'identifier les cancérogènes potentiels avant que les travailleurs et la population en général ne soient exposés.

La possibilité d'effectuer des expériences à valeur prédictive est montrée par les exemples du stilboestrol, du bis (chlorométhyl)éther, du chlorure de vinyle et des sels de chrome, substances que les tests effectués sur les animaux ont signalées comme cancérogènes avant toute étude épidémiologique. Malheureusement, ces résultats ont été accueillis avec scepticisme parce qu'on estimait les méthodes d'expérimentation insuffisantes.

Il importe, pour la future politique de prévention, de prendre les mesures suivantes:

1. donner la priorité à l'expérimentation des composés déjà produits et largement répandus et de ceux qui sont sur le point d'être produits à grande échelle;
2. élaborer des modèles expérimentaux permettant de déterminer le risque cancérogène, épreuves de détection rapide notamment ;

3. Étudier les composés les plus importants au moyen d'épreuves biologiques
longue durée.

Il est donné des résultats sur l'expérimentation du chlorure de vinyle monomère
les résultats préliminaires sont mis en tableaux pour des tests sur le styrène,
acrylonitrile et le chlorure de vinylidène.

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OCCUPATIONAL CHEMICAL CARCINOGENESIS

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Tables 1 to 18

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Table 1

CAUSES OF INCREASED ONCOGENIC RISK IN HUMAN ENVIRONMENT

- I - Concentration of oncogenic agents already present in the surface of the Earth.
- II - Surfacing of oncogenic agents from the depth of the Earth.
- III - Production of new potentially oncogenic compounds by chemical and petrochemical industry.

Table 2

KNOWLEDGE AND FACTS WHICH MAKE URGENT A NEW APPROACH
TO THE PROBLEM OF INDUSTRIAL ONCOGENESIS

- I - The potentially oncogenic agents in human environment are progressively increasing.
- II - Different oncogenic agents may have additive effects.
- III - Changes produced by oncogenic agents are largely irreversible.
- IV - Oncogenic agents may exert their effects on different organs and tissues and widely affect the target organs.
- V - A large part of the natural history of tumours take place without any clinically and sometimes otherwise detectable pathological changes.
- VI - Cancer is not a reversible disease.

Table 3
Experiments B21 and B26: Exposure by inhalation to VC in air, at 10,000, 10,000, 6,000, 2,500, 500, 250, 50 ppm.,
4 hrs. daily, 5 days weekly, for 52 weeks.
Results after 135 weeks (end of the experiments).

GROUPS AND TREATMENT	ANIMALS (SPRAGUE-DAWLEY RATS)		ANIMALS WITH TUMORS																Total (z)
	Total	Corrected number (a)	Zybal gland carcinomas (b)			Nephroblastomas (c)			Angiosarcomas Liver (d)			Other sites	Subcuta- neous sar- gomas	Skin car- cinomas	Hepatoma	Brain neu- roblastoma	Vaginary carcinoma	Other type site (n)	
			No.	%	Average latency time (weeks)	No.	%	Average latency time (weeks)	No.	%	Average latency time (weeks)								
VC 10,000 ppm. I	60	60	35	58	43	-	-	-	18	30	53	1 (e)	-	-	-	-	-	27 (e)	51
VC 10,000 ppm. III	69	61	16	26	50	5	8	59	9	15	64	3 (f)	4	3	1	7	3	4 (n)	38
VC 6,000 ppm. IV	72	60	7	12	62	4	7	65	13	22	70	3 (g)	3	1	1	3	-	8 (q)	31
VC 2,500 ppm. V	74	59	2	3	33	6	10	74	13	22	76	3 (h)	3	1	2	5	1	3 (r)	32
VC 500 ppm. VI	67	58	4	7	79	4	7	83	7	12	81	2 (i)	1	1	3	-	1	3 (s)	22
VC 250 ppm. VII	67	58	-	-	-	6	10	80	4	7	70	2 (j)	-	4	-	-	1	2 (t)	16
VC 50 ppm. VIII	64	59	-	-	-	1	2	135	1	2	135	1 (m)	1	1	-	-	2	7 (u)	10
No treatment	68	56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10 (v)	6
Total	541	475	64	-	-	26	-	-	65	-	15	12	11	7	15	8	64	206	

- a) Alive animals after 24 weeks, when the first tumour (a Zybal gland carcinoma) was observed. The percentages are referred to the corrected number.
b) Metastases to lung.
c) Metastases to liver, lung, spleen and brain.
d) Metastases to lung.
e) 1 lung angiosarcoma.
f) 1 intrabdominal angiosarcoma (next to liver); 1 angiosarcoma of the lip; 1 angiosarcoma of the nose.
g) 1 angiosarcoma in subcutaneous fibrous angiom.; 1 ossifying paravascular angiosarcoma; 1 intrabdominal angiosarcoma (next to liver).
h) 1 ossifying angiosarcoma of neck; 2 intrabdominal angiosarcomas (1 next to spleen and 1 next to ovary).
i) 1 angiosarcoma of uterus; 1 lung angiosarcoma.
j) 1 intrabdominal angiosarcoma (next to spleen); 1 intrathoracic ossifying angiosarcoma.
k) 1 intrabdominal diffused angiosarcoma.
l) Several cases of breast fibroadenomas, adrenal and pituitary tumours (generally adenomas) have not been considered, since their distribution in the different groups does not vary.
m) 7 Zybal gland adenomas; 1 skin squamous carcinoma; 1 subcutaneous fibrosarcoma; 2 mammary carcinomas; 1 hepatic angioma; 1 hepatoma; 1 ovarian angioma; 1 forestomach papilloma; 1 brain neuroblastoma; 1 Harderian gland carcinoma.
n) 2 Zybal gland adenomas; 1 ovarian cystadenocarcinoma; 1 neurilemmoma.
o) 4 Zybal gland adenomas, 2 hepatic and 1 peritoneal angiomas; 1 salivary gland adenocarcinoma.
p) 1 Zybal gland adenoma; 2 spermatocarcinomas.
q) 1 pulmonary fibrosarcoma; 2 lymphomas.
r) 1 Zybal gland adenoma; 1 lymphoma.
s) 3 Zybal gland adenomas, 1 subcutaneous angiosarcoma, 3 uterine adenocarcinomas (1 with sarcomatous component).
t) 1 invasive sarcoma of Zybal gland; 1 subcutaneous fibrosarcoma; 2 peritoneal fibrosarcomas; 2 uterine adenocarcinomas (1 with sarcomatous component).
u) 1 uterine leiomyosarcoma; 1 ovarian fibrosarcoma; 1 pulmonary rhabdomyosarcoma; 1 lymphoma.
v) Several animals with 2 or more tumours.

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- g) 1 angiosarcoma of the lips; 1 angiosarcoma of the nose.
 h) 1 ossifying angiosarcoma of neck; 1 ossifying perivascular angiosarcoma; 1 intrabdominal angiosarcoma (next to liver).
 i) 1 angiosarcoma of uterus; 1 lung angiosarcoma.
 j) 1 intrabdominal angiosarcoma (next to spleen); 1 intrathoracic ossifying angiosarcoma.
 k) 1 intrabdominal diffuse angiosarcoma.
 l) Several cases of breast fibroadenomas, adrenal and pituitary tumours (generally adenomas) have not been considered, since their distribution in the different group does not vary.
 m) 7 lymphatic gland adenomas; 1 skin squamous carcinoma; 1 subcutaneous fibrosarcoma; 2 mammary carcinomas; 1 hepatic angiosarcoma; 1 hepatoma; 1 ovarian angiosarcoma; 1 forestomach papilloma; 1 brain neuroblastoma; 1 Harderian gland carcinoma.
 n) 2 lymphatic gland adenomas; 1 ovarian cystadenocarcinoma; 1 neurilemma.
 o) 4 lymphatic gland adenomas, 2 hepatic and 1 peritoneal angiosarcoma; 1 salivary gland adenocarcinoma.
 p) 1 lymphatic gland adenoma, 2 appendicomas.
 q) 1 pulmonary fibrosarcoma, 2 lymphomas.
 r) 1 lymphatic gland adenoma; 1 lymphoma.
 s) 3 lymphatic gland adenomas; 1 subcutaneous angiosarcoma, 3 uterine adenocarcinomas (1 with sarcomatous component).
 t) 1 invasive sarcoma of lymphatic gland; 1 subcutaneous fibrosarcoma; 2 parietal fibrosarcomas; 2 uterine adenocarcinomas (1 with sarcomatous component); 1 uterine leiomyosarcoma; 1 ovarian fibrosarcoma; 1 pulmonary rhabdomyosarcoma; 1 lymphoma.
 z) Several animals with 2 or more tumours.

Table 4

Experiment B24: Exposure by inhalation to VC in air at 10,000, 6,000, 2,500, 500, 250, 50 ppm., for 4 hrs. daily, 5 days weekly, for 30 weeks.
 Results after 81 weeks (end of the experiment).

GROUPS AND TREATMENT	ANIMALS (SWISS MICE)										ANIMALS WITH TUMOURS										
	Total		Corrected number (a)				Lung tumours (b)		Mammary carcinomas (c)				Liver an- giosarcomas		Vascular tumours of other type and/or site		Epithelial tumours of the skin		Other type and/or site		Total (n)
			No.	%	Average latency time (weeks)	No.	%	Average latency time (weeks)	No.	%	No.	No.	No.	No.	No.						
	0 ^a	q														q	q	q	No.	%	No.
VC 10,000 ppm.	30	30	60	22	28	50	35	70	36	13	47	31	8	9 (a)	3 (m)	2 (p)	36				
VC 6,000 ppm.	30	30	60	26	28	54	38	70	36	8	28	33	5	9 (a)	6 (a)	4 (a)	39				
VC 2,500 ppm.	30	30	60	23	30	53	30	57	43	9	30	35	11	12 (f)	3 (e)	2 (s)	31				
VC 500 ppm.	30	30	60	29	29	58	36	66	41	7	24	37	11	18 (g)	1 (u)	1 (u)	43				
VC 250 ppm.	30	30	60	29	29	58	33	57	45	11	32	39	11	22 (h)	2 (q)	3 (v)	40				
VC 50 ppm.	30	30	60	27	30	57	2	3.5	51	12	33	43	1	13 (i)	-	1 (w)	15				
No treatment	80	70	150	74	67	141	8	6	44	-	-	-	-	1 (l)	-	7 (x)	11				
Total	260	250	510	230	241	471	184	-	60	-	-	47	84	15	20	22	220				

- a) Alive animals after 16 weeks, when the first tumour (a mammary carcinoma) was observed. The percentages are referred to the corrected number.
 b) Adenomas, some of which undergoing malignant transformation.
 c) In females. Adenocarcinomas frequently with areas of squamous metaplasia.
 d) 3 subcutaneous angiosarcomas; 4 liver fibrosarcomas; 1 heart fibrosarcoma; 1 ossifying interscapular angiosarcoma.
 e) 1 subcutaneous angiosarcoma; 2 liver fibrosarcomas; 4 liver angiosarcomas; 1 renal fibrosarcoma; 1 thyroid angiosarcoma.
 f) 3 subcutaneous angiosarcomas; 1 subcutaneous angiosarcoma; 3 liver angiosarcomas; 2 intrabdominal angiosarcomas; 2 renal angiosarcomas; 1 lung angiosarcoma.
 g) 2 subcutaneous angiosarcomas; 1 subcutaneous fibrosarcoma; 1 subcutaneous angiosarcoma; 4 liver fibrosarcomas; 1 liver angiosarcoma; 3 intrabdominal angiosarcomas; 1 intrabdominal fibrosarcoma; 1 renal angiosarcoma; 1 testicular fibrosarcoma; 1 angiosarcoma of the osseous; 1 lung fibrosarcoma; 1 lung angiosarcoma.
 h) 1 subcutaneous angiosarcoma; 6 liver fibrosarcomas; 10 liver angiosarcomas; 2 intrabdominal angiosarcomas; 1 ovarian angiosarcoma; 1 adrenal angiosarcoma; 1 lung angiosarcoma.
 i) 1 subcutaneous angiosarcoma; 1 subcutaneous fibrosarcoma; 2 subcutaneous angiosarcomas; 2 liver fibrosarcomas; 3 liver angiosarcomas; 1 intrabdominal angiosarcoma; 1 ovarian angiosarcoma; 1 intrathoracic fibrosarcoma; 1 angiosarcoma of the interscapular fat pad.
 j) 1 angiosarcoma of uterus.
 k) 2 squamous carcinomas; 1 invasive squamous carcinoma.
 l) 5 squamous carcinomas; 1 squamous carcinoma.
 m) 1 squamous carcinoma; 2 squamous carcinomas.
 n) 1 squamous carcinoma.
 o) 1 skin adenocarcinoma; 1 basaloma.
 p) 1 lymphatic gland adenoma; 1 forestomach papilloma.
 q) 1 subcutaneous leiomyosarcoma; 1 forestomach papilloma; 1 Harderian gland adenoma; 1 lymphoma.
 r) 1 forestomach papilloma; 1 parotid gland mixed tumour.
 s) 1 lymphatic gland adenoma.
 t) 1 lymphatic gland adenoma; 1 Leydig cell tumour; 1 lymphoma.
 u) 1 parotid gland adenocarcinoma.
 v) 1 subcutaneous leiomyosarcoma; 1 adenoma of colon; 1 ovarian carcinoma; 1 leiomyosarcoma of uterus; 3 lymphomas.
 w) 1 parotid gland adenocarcinoma.
 x) Several cases with 2 or more tumours.

Table 5

Experiment B78 : Exposure by inhalation to VC in air at 10,000, 6,000, 2,500, 500, 250, 50 ppm., 4 hrs. daily, 5 days weekly, for 30 weeks.
Results after 105 weeks.

GROUPS AND TREATMENT	ANIMALS (GOLDEN HAMSTERS)		ANIMALS WITH TUMOURS						
	Total	Survivors	Liver angiosarcomas	Skin trichoeplitheliomas and basalomas (a)	Melanomas	Lymphomas	Fore stomach epithelial tumours (b)	Other type and/or mixts	Total (g)
			No.	No.	No.	No.	No.	No.	No.
I									
VC 10,000 ppm.	35	-	-	6	1	-	4	2 (c)	4
II									
VC 6,000 ppm.	32	-	1	2	2	2	7	7 (d)	10
III									
VC 2,500 ppm.	33	-	-	1	-	1	10	3 (e)	12
IV									
VC 500 ppm.	33	-	2	3	-	1	7	2 (f)	12
V									
VC 250 ppm.	32	-	-	3	-	1	2	-	6
VI									
VC 50 ppm.	33	-	-	6	1	1	4	-	10
VII									
No treatment	70	1	-	2	-	2	-	-	5
Total	268	1	3	23	4	8	34	14	59

- a) Several cases with acanthosis and some undergoing malignant transformation.
b) Papillomas, acanthomas, some of which undergoing malignant transformation.
c) 1 subcutaneous angiosarcoma; 1 gall-bladder adenocarcinoma.
d) 2 hepatomas; 2 liver fibrosarcomas; 2 liver angiosarcomas; 1 biliary duct adenocarcinoma.
e) 1 hepatoma; 1 liver fibrosarcoma; 1 liver angiosarcoma.
f) 1 subcutaneous angiosarcoma; 1 bronchial carcinoma.
g) Several animals with 2 or more tumours.

Table 6

Experiment B79 : Exposure by ingestion (stomach tube) to VC in olive oil, at 50.00, 16.65, 3.33 mg/Kg body weight, once daily, 4-5 days weekly, for 52 weeks.
Results after 84 weeks.

GROUPS AND TREATMENT	ANIMALS (SPRAGUE-DAWLEY RATS)		ANIMALS WITH TUMOURS					
	Total	Survivors	Zymbal gland carcinomas	Nephroble- stomas	Angiosarcomas		Other type and/or mixt (b)	Total
					Liver	Other sites		
			No.	No.	No.	No.	No.	No.
I								
VC 50.00 mg/Kg	80	25	-	-	8	1 (a)	3 (c)	11 (d)
II								
VC 16.65 mg/Kg	80	33	1	1	5	-	-	7
III								
VC 3.33 mg/Kg	80	39	-	-	-	-	-	-
IV								
Control olive oil	80	37	-	-	-	-	-	-
Total	320	134	1	1	13	1	3	18

- a) 1 thymus angiosarcoma.
b) Several cases of breast fibroadenomas, adrenal and pituitary tumours (generally adenomas) have not been considered, since their distribution in the different groups does not vary.
c) 1 mammary carcinoma, 2 forestomach papillomas.
d) 1 animal with 2 tumours.

000, 5,000, 2,500,
daily, for 30 weeks.

H TUMOURS			
Forestomach epithelial tumours (b)	Other type and/or site	Total (g)	
No.	No.	No.	
4	2 (e)	4	
7	7 (d)	10	
10	3 (a)	12	
7	2 (f)	12	
2	-	6	
4	-	10	
-	-	5	
34	14	59	

on.

1.

carcinoma.

C in olive oil,
once daily,

TUMOURS		
Uterine tumours Other sites	Other type and/or site (b)	Total
No.	No.	No.
1 (a)	3 (c)	11 (d)
-	-	7
-	-	-
-	-	-
1	3	18

(generally adenomas) have
does not vary.

Table 7

Experiment B15 : Exposure by inhalation to VC in air at 10,000 and 5,000 ppm.
of breeders, 4 hrs. daily, for 1 week (from 12th to 18th day
of pregnancy).
Results after 143 weeks (end of the experiment).

GROUPS AND TREATMENT	ANIMALS (SPRAGUE-DAWLEY RATS)		ANIMALS WITH TUMOURS					Total
	Total	Survivors	Zymbal gland carcinomas	Nephroblas- tomas	Angiosarcomas		Other type and/or site	
			No.	No.	Liver	Other sites	No.	
I VC 10,000 ppm. Breeders	30	-	1	-	-	1 (a)	2 (d)	3
II VC 5,000 ppm. Breeders	30	-	-	-	-	-	-	-
III VC 10,000 ppm. Offspring	54	-	3	1	-	2 (b)	2 (e)	8
IV VC 5,000 ppm. Offspring	32	-	1	-	-	2 (c)	4 (f)	6
Total	146	-	5	1	-	5	8	17

- a) 1 intraductal angiosarcoma.
b) 1 subcutaneous angiosarcoma; 1 angiosarcoma of the leg.
c) 1 subcutaneous angiosarcoma; 1 intraductal angiosarcoma.
d) 1 liver fibrosarcoma; 1 liver angioma.
e) 1 Zymbal gland fibrosarcoma; 1 ovarian leiomyosarcoma.
f) 1 Zymbal gland adenoma; 1 skin carcinoma; 1 subcutaneous fibrosarcoma; 1 mammary carcinoma.

Table 8

TUMOURS PRESENTLY CORRELATED TO VC EXPOSURE (BY INHALATION), ON EXPERIMENTAL RODENTS AND MAN

Species	TUMOURS									
	Angiosar- comas of liver	Tumours of brain	Tumours of lung	Lympho- mas and leuke- mias	Angiosar- comas and an- giomas of other sites	Nephro- blasto- mas	Sebaceous cutaneous carcino- mas	Squamous tumours of epi- dermis	Lam- ary car- cinomas	Hepa- to- mas
Rat	+	+			+	+	+	+	(+)	+
Mouse	+		+		+		+	+	+	+
Hamster	+		(+)	(+)	(+)			+		(+)
Man	+	(+)	(+)	(+)						(+)

Table 9

Experiment B210 : Exposure by inhalation to VC for 100 hrs., following different schedules.
Results after 59 weeks.

GROUPS AND TREATMENT	ANIMALS (SPRAGUE-DAWLEY RATS)		ANIMALS WITH TUMOURS					
	Total	Survivors	Zymbal gland carcinomas	Nephroblg stomas	Angiosarcomas		Other type and/or site	Total
			No.	No.	Liver	Other sites		
I VC 10,000 ppm. 4hd/5dw/25w	120	84	4	-	-	-	-	4
II VC 6,000 ppm. 4hd/5dw/25w	120	85	3	-	-	-	1 (b)	4
III VC 10,000 ppm. 1hd/4dw/25w	120	89	4	-	-	-	-	4
IV VC 6,000 ppm. 1hd/4dw/25w	120	85	-	-	-	1 (a)	-	1
V VC 10,000 ppm. 4hd/1dw/25w	120	96	2	-	-	-	1 (c)	3
VI VC 6,000 ppm. 4hd/1dw/25w	120	97	-	-	-	-	1 (d)	1
VII No treatment	249	180	-	-	-	-	-	-
Total	969	716	13	-	-	1	3	17

- a) 1 subcutaneous angiosarcoma.
b) 1 lymphoma.
c) 1 subcutaneous angioepithelioma.
d) 1 lymphoma.

VC
VC
VC
VC
No treat
Total

Table 10

Experiment B29 : Exposure by inhalation to VC in air at 50 ppm., 4 hrs. daily,
5 days weekly, for 52 weeks.
Results after 59 weeks.

GROUPS AND TREATMENT	ANIMALS (SPRAGUE-DAWLEY RATS)		ANIMALS WITH TUMOURS					
	Total	Survivors	Zymbal gland carcinomas	Nephroblg stomas	Angiosarcomas		Other type and/or site	Total
			No.	No.	Liver	Other sites		
I VC 50 ppm.	300	216	1	-	-	-	-	1
II No treatment	100	72	-	-	-	-	-	-
Total	400	288	1	-	-	-	-	1

GROUPS AND TREATMENT
I VC 50 ppm.
II No treat
Total

, following different schedules.

RATS WITH TUMOURS			
Angiosarcomas		Other type and/or site	Total
Liver	Other sites		
No.	No.	No.	No.
-	-	-	4
-	-	1 (b)	4
-	-	-	4
-	1 (a)	-	1
-	-	1 (c)	3
-	-	1 (d)	1
-	-	-	-
-	1	3	17

Table 11

Experiment BT15 : Exposure by inhalation to VC in air, at 25, 10, 5, 1 ppm., 4 hrs. daily, 5 days weekly, for 52 weeks.
Results after 46 weeks.

GROUPS AND TREATMENT	ANIMALS (SPRAGUE-DAWLEY RATS)		ANIMALS WITH TUMOURS					
			Zymbal gland carcinomas	Nephroblastomas	Angiosarcomas		Other type and/or site	Total
	Total	Survivors	No.	No.	Liver	Other sites		
I								
VC 25 ppm.	120	110	-	-	-	-	-	-
II								
VC 10 ppm.	120	111	-	-	-	-	-	-
III								
VC 5 ppm.	120	113	-	-	-	-	-	-
IV								
VC 1 ppm.	120	112	-	-	-	-	-	-
V								
No treatment	120	97	-	-	-	-	-	-
Total	600	543	-	-	-	-	-	-

Table 12

Experiment BT17 : Exposure by inhalation to VC in air at 1 ppm., 4 hrs. daily, 5 days weekly, for 52 weeks.
Results after 46 weeks.

GROUPS AND TREATMENT	ANIMALS (WISTAR RATS)		ANIMALS WITH TUMOURS					
			Zymbal gland carcinomas	Nephroblastomas	Angiosarcomas		Other type and/or site	Total
	Total	Survivors	No.	No.	Liver	Other sites		
I								
VC 1 ppm.	120	96	-	-	-	-	-	-
II								
No treatment	130	89	-	-	-	-	-	-
Total	250	185	-	-	-	-	-	-

RATS WITH TUMOURS			
Angiosarcomas		Other type and/or site	Total
Liver	Other sites		
No.	No.	No.	No.
-	-	-	1
-	-	-	-
-	-	-	1

Table 13

Experiment BT27 : Exposure by ingestion (stomach tube) to VC in olive oil,
at 1, 0.3, 0.03 mg/kg body weight, once daily, 4-5 days
weekly, for 104 weeks.
Results after 21 weeks.

GROUPS AND TREATMENT	ANIMALS (SPRAGUE-DAWLEY RATS)		ANIMALS WITH TUMOURS					
	Total	Survivors	Zymbal gland carcinomas	Nephrobl stomas	Angiosarcomas		Other type and/or site	Total
			No.	No.	Liver	Other sites		
I VC 1 mg/kg/bw	158	147	-	-	-	-	-	-
II VC 0.3 mg/kg/bw	159	145	-	-	-	-	-	-
III VC 0.03 mg/kg/bw	163	147	-	-	-	-	-	-
IV Control: olive oil	159	148	-	-	-	-	-	-
Total	639	587	-	-	-	-	-	-

Table 14

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VC 0.03 mg/Kg/bw	163	147	-	-	-	-	-	-
IV Control: olive oil	159	148	-	-	-	-	-	-
Total	639	587	-	-	-	-	-	-

Table 14

Experiments B012 and B12001 ; Treatment: 1 subcutaneous injection of 30 mg of the compound in 1 cc of H₂O.

Groups and Treatment	Animals (1) (Sprague-Dawley rats)		Tumours (sarcomas at the site of injection)			Ended Experiments length (weeks)	Ongoing experiments time from start (weeks)
	Total	Survivors	No. of animals with tumours	Average latency time (weeks)	Histology type		
I Chromite	40	-	-	-	-	133	
II Neochromium	40	-	9	78	Rhabdomyosarcomas and fibrosarcomas	149	
III Chromium allumen	40	-	8	72	Rhabdomyosarcomas and fibrosarcomas	137	
IV Chromium yellow (Lead chromate)	40	-	26	40	Rhabdomyosarcomas and fibrosarcomas	150	
V Chromium orange (Lead chromate)	40	-	27	47	Rhabdomyosarcomas and fibrosarcomas	150	
VI Molybdenum orange (Lead chromate, sulphate and molybdate)	40	-	36	32	Rhabdomyosarcomas and fibrosarcomas	117	
VII Cadmium yellow (Cadmium sulphide)	40	-	16	86	Rhabdomyosarcomas and fibrosarcomas	143	
VIII Iron yellow (Iron oxide)	40	-	1	77	Rhabdomyosarcomas and fibrosarcomas	125	
IX Iron red (Iron oxide)	40	-	-	-	-	134	
X Control	140	-	-	-	-	127	
XI Titanium oxide	120	115	-	-	-		35
XII Zinc chromate	80	80	-	-	-		4

(1) 50% males, 50% females

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Table 15

PLAN OF THE EXPERIMENTS ON THE BIOLOGICAL EFFECTS OF STYRENE

Experiments	Species and age at start (weeks)	Groups	No. of animals at start			Treatment					Weeks from start
			Total	♂	♀	Route	Dose	Stopped	Ongoing	Length (weeks)	
BT101	Rats (13)	I	60	30	30	Inhalation	300 ppm		+	44	44
		II	60	30	30	"	200 ppm		+	44	44
		III	60	30	30	"	100 ppm		+	44	44
		IV	60	30	30	"	50 ppm		+	44	44
		V	60	30	30	"	25 ppm		+	44	44
		VI	120	60	60	Untreated (controls)	-				44
		Total	420	210	210						
BT102	Rats (13)	I	80	40	40	Ingestion	250 mg/kg in olive oil		+	37	37
		II	80	40	40	"	50 mg/kg in olive oil		+	37	37
		III	80	40	40	"	control: olive oil		+	37	37
		Total	240	120	120						
BT103	Rats (13)	I	80	40	40	4 intrabdominal injections	50 mg in olive oil (x 4)	+			38
		II	80	40	40	"	control: olive oil	+			38
		Total	160	80	80						
BT104	Rats (13)	I	80	40	40	1 subcutaneous injection	50 mg in olive oil	+			38
		II	80	40	40	"	control: olive oil	+			38
		Total	160	80	80						
TOTAL				980	490	490					

Table 16

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BT103	Rats (13)	I	80	40	40	4 intrabdominal injections	50 mg in olive oil (x 4)	+			38
		II	80	40	40	"	control:olive oil	+			38
		Total	160	80	80						
BT104	Rats (13)	I	80	40	40	1 subcutaneous injection	50 mg in olive oil	+			38
		II	80	40	40	"	control: olive oil	+			38
		Total	160	80	80						
TOTAL			960	490	490						

Table 16

PLAN OF THE EXPERIMENTS ON THE BIOLOGICAL EFFECTS OF ACRYLONITRILE

Experiments	Species and age at start (weeks)	Groups	No. of animals at start			Treatment					Weeks from start
			Total	♂	♀	Route	Dose	Stopped	Ongoing	Length (weeks)	
BT201	Rats (12)	I	62	32	30	Inhalation	40 ppm		+	35	35
		II	60	30	30	"	20 ppm		+	35	35
		III	60	30	30	"	10 ppm		+	35	35
		IV	60	30	30	"	5 ppm		+	35	35
		V	60	30	30	Untreated (controls)	-				35
		Total	302	152	150						
BT202	Rats (12)	I	105	58	47	Ingestion (1)	20 mg/kg in olive oil	+		6	35
		II	80	40	40	"	control: olive oil	+		6	35
		Total	185	98	87						
BT203	Rats (10)	I	82	42	40	Ingestion (2)	5 mg/kg in olive oil		+	21	21
		II	158	82	76	"	control: olive oil		+	21	21
		Total	240	124	116						
TOTAL											
			727	374	353						

(1) 3-5 times weekly

(2) 3 times weekly

Table 17

PLAN OF THE EXPERIMENTS ON THE BIOLOGICAL EFFECTS OF VINYLIDENE CHLORIDE

Experiments	Species and age at start (weeks)	Groups	No. of animals at start			Treatment					Weeks from start
			Total	♂	♀	Route	Dose	Stopped	Ongoing	Length (weeks)	
BT401	Rats (16)	I	120	60	60	Inhalation	150 ppm		+	21	21
		II	60	30	30	"	100 ppm		+	21	21
		III	60	30	30	"	50 ppm		+	21	21
		IV	60	30	30	"	25 ppm		+	21	21
		V	60	30	30	"	10 ppm		+	21	21
		VI	200	100	100	Untreated (controls)	-				21
		Total	560	280	280						
BT402	Mice (16) (9)	I	120	60	60	Inhalation	200 ppm	+		2 days	21
		II	60	30	30	"	100 ppm	+		2 days	21
		III	60	30	30	"	50 ppm	+		4 days	21
		IV	60	30	30	"	25 ppm		+	21	21
		V	240	120	120	"	25 ppm		+	19	19
		VI	60	30	30	"	10 ppm		+	21	21
		VII	200	100	100	Untreated (controls)	-				21
		Total	800	400	400						
BT403	Rats (9)	I	100	50	50	Ingestion	20 mg/kg in olive oil		+	32	32
		II	100	50	50	"	10 mg/kg in olive oil		+	32	32
		III	100	50	50	"	5 mg/kg in olive oil		+	32	32
		IV	200	100	100	"	control:olive oil		+	32	32
		Total	500	250	250						
BT404	Rats (9)	I	100	50	50	Ingestion	0.5 mg/kg in olive oil		+	21	21
BT405	Chinese Hamsters (28)	I	60	30	30	Inhalation	25 ppm		+	13	13
		II	35	18	17	Untreated (controls)	-				13
		Total	95	48	47						
TOTAL			2,055	1,029	1,026						

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	(9)	II	100	50	50	"	olive oil		+	32	32
		III	100	50	50	"	10 mg/kg in olive oil		+	32	32
		IV	200	100	100	"	5 mg/kg in olive oil		+	32	32
		Total	500	250	250		control: olive oil		+	32	32
BT404	Rats (9)	I	100	50	50	Ingestion	0.5 mg/kg in olive oil		+	21	21
BT405	Chinese Hamsters (28)	I	60	30	30	Inhalation	25 ppm		+	13	13
		II	35	18	17	Untreated (controls)	-				13
		Total	95	48	47						
TOTAL			12,055	1,028	1,027						

Table 18

Experiment BT1001 : Treatment: 1 subcutaneous injection of 2 mg of Adriamycin in 1 cc of olive oil.
Results : after 38 weeks.

GROUPS AND TREATMENT	ANIMALS (SPRAGUE-DAWLEY RATS)				TUMOURS (1)		AVERAGE LATENCY TIME (weeks)	
	Sex	Number	Corrected number	Survivors	No.	%	First tumour	Average
I Adriamycin 2 mg	♀	42	42	35	14	35	19	31
	♂	40	40	30	12	30	25	32
II Control: olive oil	♀	40	40	34	-	-	-	-
	♂	40	39	30	-	-	-	-
Total	♀ ♂	162	161	129	26			

(1) At the site of injection.

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