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# Insect Chemosterilants

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The review covers the literature data on the application of chemosterilants in the fight against harmful insects. The chemosterilants have been classified on the basis of their structural characteristics.
The bibliography includes 174 references.

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## I. INTRODUCTION

The constantly rising requirement for food products to support the rapidly growing population of the earth necessitates an intensive application of chemical agents for the protection of plants in order to increase crop yields. However, large-scale application of chemical poisons has clearly shown both advantages and disadvantages of the chemical methods of combating pests. Without dealing with the undoubted advantages of this method, we shall only mention some of the problems which have arisen in consequence of the intensive application of pesticides. These include in the first place the accumulation of residual chemical poisons and their metabolites in the soil and plant, animal, and human tissues.

A progressive development of resistance to chemical poisons (in particular organic chloro-derivatives) has been noted in an increasing number of harmful species of insects and ticks (mites). The use of stable preparations with a wide spectrum of action upsets biocenotic equilibria, leads to the destruction of useful insects and ticks (mites), and gives rise to explosive multiplication of various pests.

These undesirable consequences of the large scale and intensive application of chemical poisons led to an increase of interest in many countries (particularly in the post-war years) in the biological methods of combating pests. There has been a sharp increase in publications on this question: 308 in 1955 [1] and 578 in 1958.[2] For example, in 1955 66% of all investigations in the USA were devoted to the application of insecticides and in 1967 only 21%, while the percentages of the studes dealing with biological and other specific methods were 17 and 42%.[3]

There has been a greater expansion of investigations into the ecology, biology, and dynamics of the populations of pests and entomophages and their biocenotic interactions, genetics and cytogenetics of insects, large scale cultivation of entomophages on artificial media, etc. However, it is as yet impossible to abandon the chemical methods of combating pests and employ only ecological and biological procedures. The most promising technique appears to involve a planned combination of all methods of combating pests with the maximum limitation of the use of highly toxic and stable pesticides. Such a system has been called the integral method (or the integrated system) of plant protection. Apart from taking into account the structure of agrobiocenoses, this includes the introduction of types of cultivated plants resistant to pests and disease, the prediction of the number of harmful species, and the use of selective chemical poisons, biological preparations, antibiotics, and herbicides. This programme should also incorporate the application of attractants, repellants, and chemical sexual sterilising agents, and also the breeding of entomophagous insects (or phytophagous insects in the fight against weeds) and sterile insects. The ultimate aim of integrated plant protection is the arrest or sharp reduction of the rate of "biological reconstruction", i.e. the invasion of cultivated areas by weeds, disease, and pests[4]. As regards harmful insects, this result can be achieved by methods in the following two groups: (a) by acting on the insects and the environment (destruction, modification of behaviour, destruction of natural resources); (b) by acting on hereditary properties (sexual sterilisation, introduction of harmful genes into the population)[5].

This review deals with methods of combating pests based on chemical sexual sterilisation.

Sexual sterilisation is so far the only method developed up to the stage of practical application in which the fight against pests involves influencing the hereditary properties of the species, Treatment of the population by an insecticide may be effective only when the increase of "standard mortality" is much greater than the "level of the standard birthrate". However, in accordance with the feedback principle, the temporary increase in the "rate of mortality" is countered by the insects by an increase of the "standard birthrate" and the initial population density is restored[6]. Moreover, the effectiveness of pesticides falls with decreasing population density of the pests. The fight against pests by the sterilisation method is based on another principle. The level of the "rate of mortality" remains temporarily unchanged, while the "birth rate" in the population is reduced because a definite percentage of its members are sterile—the population density will continue to decrease.

The increased interest of entomologists in the sexual sterilisation of insects can also be explained by the fact that this method introduces fundamentally new possibilities into the practice employed in combating harmful insects. Firstly, there is the high effectiveness of the method. Knipling[7] calculated the relative effectiveness of chemical methods of combating pests and of chemical sexual sterilisation. In the version where insecticides were employed (technical efficiency 95%) the insects surviving after treatment (5% of the initial population) multiplied and, possessing a biotic potential increased by a factor of five, restored towards the third generation the initial population density. In the version where a chemical sterilising agent was employed, all the insects survived but 95% became sterile.

These, by mating with one another and also with the remaining fertile individuals, deprived the latter of the possibility of multiplying. Since the sterile insects predominate in the population (19:1), then evidently only 5% of the cases of mating will be between fertile insects, while in all remaining cases (95%) one of the partners will be sterile. Thus only 0.25% of the individuals in the initial population will multiply. Therefore the number of insects in the succeeding generations falls sharply and towards the third is already virtually zero.

The calculations described are based on data obtained in the treatment of populations under natural conditions. Similar results are obtained when insects which have been trapped or bred under laboratory conditions are released among the natural population after treatment with the chemosterilant. It is then only necessary to maintain the required ratio between the sterile and natural insects.

Such high effectiveness permits the expectation that the population of many pests will be rapidly reduced and, in the case of some species residing in isolated centres, will be completely eradicated.

The second factor is the high specificity of the method. When the procedure involving the release of sterile insects or the application of chemosterilants in combination with sexual attractants is employed, the operation of the method is restricted to the species against which the fight is directed. The use of sexual sterilisation in combination with light traps or food attractants (or agents stimulating egg laying) reduces somewhat the specificity to group specificity but nevertheless it is still quite high and useful species of the biocenosis are not involved. The direct application of chemosterilants under natural conditions requires the utilisation of selective preparations of low toxicity, since the treatment of a natural population with chemosterilants available hitherto approaches the chemical method in the selectivity of action on the biocenosis.

Thirdly, the use of chemosterilants permits the solution of the problem of the resistance of insects to insecticides. Under laboratory conditions no difference was observed in the sensitivity to a chemosterilant of strains of the house fly resistant and susceptible to organophosphorus insecticides[8]. A similar situation was observed for spider mites[9]. Insects resistant to organic chloro-insecticides are highly susceptible to chlorine-containing chemosterilants[10]. However, experiments have shown the possibility of the development of resistance to chemosterilants also[11]. The degree of acquired resistance is relatively low and exceeds the resistance by the population sensitive to the sterilising agent by a factor of 4–5 only, while the resistance to organic chloro-insecticides increases very markedly (by a factor of hundreds and thousands)[12]. Moreover, the development of resistance in insects is accompanied by the accumulation of "small recessive genetic defects", which leads to an increase of sensitivity to the preparation after 4–5 generations[13] and the initial situation is thus restored. It is noteworthy that, when the method involving the release of sterile insects is employed, the problem of resistance to chemosterilants does not arise at all.

Fourthly, the effectiveness of the method of sexual sterilisation increases as the population density of the pest is reduced, while the effectiveness of insecticides and the majority of entomophages falls under these conditions.

Moreover, it is noteworthy that the application of this method of combating harmful insects solves the problem of the inaccessibility of the individuals for treatment, which is particularly important for certain species with a secretive mode of living. Sterile males driven by the sexual instinct seek their partners everywhere including secluded microscopic sites normally inaccessible to treatment by insecticides.

An essential condition which must be met in the method of sexual sterilisation is the retention of the normal sexual activity and normal behavioural responses in sterilised insects, which enables them to seek normal females under natural conditions and impregnate them.

In the initial experiments sexual sterilisation was achieved by irradiating insects with ultraviolet light and X-, $\alpha$-, $\beta$-, and $\gamma$-rays. The success of this programme of eradication of the important pest *Cochliomyia hominivorax* Coq. in 1958–1959 over an extensive territory[14] stimulated research into methods for combating harmful species based on the release of sterile insects among natural populations. The studies carried out to discover more convenient and economical methods of sexual sterilisation of insects showed that certain chemicals give rise to phenomena in the insect organism similar in their final result to radiation-induced sterilisation, i.e. sexual sterility.

The sexual sterility of insect males can be caused by dominant lethal mutation in the sperm, aspermia, or the inactivation of the sperm. On the other hand, in females sterility may result from the suppression of ovogenesis or lethal mutations in egg cells.

The first experiments on the chemical sterilisation of insects were carried out in 1946–1947.[15-17] However, the ability of certain biologically active substances to cause hereditary changes (mutations) when acting upon organisms was noted in even earlier studies by Sakharov, Lobashev, and Smirnov in 1932–1933.[18]

Large scale research on chemosterilants was begun in 1960 in the USA and in 1961 several compounds with sterilising activity were discovered. During the next three years, more than 3000 compounds were tested, of which only about 100 preparations proved to be effective as sexual chemosterilants[19,20]. Up to the present time, the number of tested preparations has increased greatly: one laboratory in Gainsville (Florida, USA) alone tests annually 500–700 compounds in order to elucidate their sterilising activity, and Bořkovec's compilation[6] gives a list of 483 effective preparations tested on 75 insects species and 7 tick species.

The number of publications on chemical sterilisation alone increases annually in a geometrical progression and in 1967 amounted to 400 papers[21]. At present the bibliography on this subject includes more than 600 items. Several reviews of the literature on the problems of chemical sterilisation have been published: by Rukavishnikov[12,22-25], Bořkovec[6,19,26], Smith[27,28], Bertram[29], Kilgore[30], a group of authors under the editorship of La Brecque and Smith[21], and Proverbs[31].

The essential feature of the method of chemosterilants consists in the treatment of specially bred or trapped insects with specific chemical mutagenic agents (chemosterilants), followed by the release of the insects among the natural population or alternatively the natural insect population is treated at sites where it is concentrated.

However, later a distinction was drawn between chemical mutagenic and chemosterilants[32]. Many chemical mutagenic agents are sexual sterilising agents at the same time but not all chemosterilants can cause inheritable mutations or cytogenetic changes.

Chemosterilants may be defined as compounds which reduce or completely eliminate the capacity of animals for reproduction. There exist several groups of substances capable of affecting the reproductive functions of the species. The most thoroughly investigated are substances

which inhibit the ovogenetic and spermatogenetic processes, destroying ripe egg cells and sperm [33,34] or causing dominant lethal mutations in the chromosomal apparatus of the sexual cells. Under these conditions, the egg cells survive and the sperm remain mobile, but the zygote dies in the early stages of development. These substances can be defined as chemosterilants in a narrow sense of the word.

In addition, there exists a group of substances which prevent mating by frightening the males away from the females or acting in a somewhat different way on the copulation process [12]. Certain compounds are capable of either eliminating or masking the odour of sexual attractans, which among insects serves as a guide in the search for a mate [12]. It may be expected that some of the substances will act on the insect organism in at least two ways. Data have been published showing that females of the codling moth treated with chemosterilants belonging to the first group or irradiated attracted males less then the untreated specimens [35,36]. Possibly, in addition to the expected effect (sexual sterilisation), chemosterilants and radiation suppressed the activity of the glands secreting the sexual attractant. Studies on these lines would be very timely, since they might provide a basis for the development of yet another method in the biological fight against harmful insects.

The inhibition of the multiplication of insects by certain chemical poisons [37] and also interference with metamorphisis and the sterility of individuals treated by the analogues of certain hormones [38,39] have been observed. However, compounds belonging to the above group (with the exception of the first) can be classified as chemosterilants, only in an extremely arbitrary sense and therefore will not be discussed in detail in the present review.

Frequently attempts at classification of chemosterilants have encountered difficulty in combining a wide variety of compounds into systems based on the similarity of differences in their structures, reactivities, or biochemical effects. In the first experiments on chemical sterilisation compounds employed in the treatment of cancer were applied [19,41-44].

An empirical relation between mitotic agents influencing the process of cell division (mitosis) and compounds with antineoplastic (antitumour) activities was established in the period between 1950 and 1960. [40] At the same time a relation was discovered between cytostatic activity and the effectiveness of these substances as chemosterilants. Although a direct correlation was not always evident, nevertheless compounds with high antineoplastic activities (TEM†. tepa, thiotepa, apholate, tretamine, and others) proved to be promising sexual sterilising agents for insects, and served as a starting point for the selection of possible chemosterilants.

Up to 1964, in fact all the compounds effective as sterilising agents for insects were either anticancer substances or chemically related compounds [19]. Therefore we adopted (with small changes) the system employed in studies on anticancer agents for the classification of chemosterilants. Initially five categories were considered: alkylating substances, antimetabolites, radiomimetic compounds, mitotic poisons, and mixed agents [45]. This classification was partly chemical and partly biological. In addition, some overlapping of categories was noted. Later [6] the system was greatly simplifed and included only three categories: alkylating agents, antimetabolites, and compounds of a mixed type. Radiomimetic and mitotic poisons have been included in one of the above three categories.

## II. ALKYLATING CHEMOSTERILANTS

Alkylation, which usually takes place when chemosterilants react with cell elements *in vivo* may be defined as the substitution of a hydrogen atom in the molecule by an alkyl group [see reaction (I)], [46,47] as the direct addition of such a group to a tertiary nitrogen atom with formation of a quaternary ammonium ion [reaction (II)], and finally as its addition to an anion, which leads to the formation of esters [reaction (III)]: [18,46]

$$R^+ + R'SH \rightarrow R'S{-}R + H^+ \quad (I)$$

$$R^+ + NR'_3 \rightarrow R'_3\overset{+}{N}{-}R \quad (II)$$

$$R^+ + O{=}\overset{O{-}R'}{\overset{|}{P}}{-}\overset{-}{O}\overset{O{-}R''}{|} \rightarrow O{=}\overset{O{-}R'}{\overset{|}{P}}{-}O{-}R\ (III)\ \ \overset{O{-}R''}{|}$$

The alkylating group R can be a hydrocarbon residue, for example the ethyl group $-CH_2CH_3$, or can contain a functional substituent, for example $-CH_2COCH_3$, $-CH_2CH_2NH_2$ (aminoalkyl), or $-CH_2CH_2COOH$.

The aminoethylation reactions of nucleoprotein structures responsible for the hereditary properties of the cell (organism) in fact determine the mutagenic activity of alkylating agents [18].

Tests on compounds belonging to the following main groups of alkylating agents (Ross's classification [46]) have been described in the literature: sulphonic acid esters, dichloroethylamines (nitrogenous analogues of yperites), aziridines, halogenoalkyl ester of sulphuric acid, phosphoric acid esters, chloromethyl ethers, sulphonium and ammonium derivatives, 2-chloroethyl sulphides, ethoxides, $\beta$-lactones, diazoalkanes, and also activated derivatives of ethylene, halogenomethyl derivatives of ketones and ethers, olefins, and alcohols. The last two groups of substances (olefins and alcohols) alkylate only at a high temperature or in the presence of specific catalysts and do not operate in the living cells of the organism [6].

Sulphonic acid esters, dichloroethylamines, and ethyleneamines proved to be the most promising among alkylating chemosterilants (more than 50% of all the effective preparations belong to these groups) and they will be examined in detail. Examples of the remaining groups have been tested as sterilants on the screw-worm fly *Cochliomyia hominivorax* Coq., but largely negative results were obtained and therefore they will not be considered further.

### Sulphonic Acid Esters

The alkylating activity of alkanesulphonic acid esters depends on the strength of the O–R′ bond. Alkane- and substituted alkylmethane-sulphonates (IV)

$$R{-}\overset{O}{\overset{\|}{S}}{-}O{-}R' \quad (IV)$$
$$\overset{O}{\|}$$

where R′ = $CH_3$, are powerful anticancer agents [6]. The data on these compounds as chemosterilants are so far

† Significance of abbreviation uncertain (Ed. of Translation).

few but some of them, for example bisulphan (V) and its dimethyl derivative (VI),

$$CH_3SO_2O-(CH_2)_4-OSO_2CH_3 \quad (V)$$

$$CH_3SO_2O-\underset{\displaystyle CH_3}{\underset{|}{CH}}(CH_2)_2\underset{\displaystyle CH_3}{\underset{|}{CH}}-OSO_2CH_3 \quad (VI)$$

proved to be highly effective sexual sterilants for certain insect species, in particular beetles[6], and their toxicity is low. Certain sulphonic acid esters are effective chemosterilants for the Callitroga [screw-worm] fly. Diesters proved to be more active than monoesters as sterilants, but their toxicity is also higher[48].

## Dichloroethylamines

In studies concerned with the treatment of cancer these compounds occupy a special place. A review of alkylating agents[49] tested as anticancer chemotherapeutic agents gives a list containing more than 20 000 compounds[49]. Chloroethylamines are structurally similar to yperite in which the sulphur atom has been replaced by nitrogen (VII):

$$CH_3-N\begin{matrix}\diagup CH_2CH_2Cl\\ \diagdown CH_2CH_2Cl\end{matrix} \quad (VII).$$

Compounds containing one 2-chloroethylamino-group ($=NCH_2CH_2Cl$) belong to single-chain yperites; compounds with a di-(2-chloroethyl)amino-group [$-N(CH_2CH_2Cl)_2$] are two-chain yperites. The presence of a number of functional groups in biological alkylating agents is regarded as an imporant property which promotes the cross-linking of chromosomes or other physiologically important cellular formations[46,50]. However, although cross-linking can sometimes account for the disproportionately high activity of polyfunctional compounds, the mechanism of the action of chloroethylamines is much more complex. Interference with metabolism (in particular of phosphorus[51]) and the inhibition of enzymes involved in the nucleic acid metabolism and also of glycolytic enzymes have been noted. This leads to the inhibition of cell division and gives rise to morphological changes in dividing chromosomes: fragmentation, mutual adhesion, the formation of bridges between chromosomes in the anaphase, and others[52].

Historically yperite derivatives have been some of the first chemical mutagenic agents discovered[15,16]. A large number of compounds of this series have already been tested as chemosterilants but only some of them showed a high effectiveness.

Nitrogenous yperite and its derivatives are effective sexual sterilants for the males of mice[53], the house fly[43], the Mexican fruit fly[54,55], and the Callitroga fly[56].

## Derivatives of Aziridine

This is the most "promising" group of alkylating compounds. More than one third of the effective chemosterilants quoted in Bořkovec's compilation[6] are aziridine (ethylene imine) derivatives (VIII):

$$\begin{matrix}H_2C-CH_2\\ \diagdown\ \diagup\\ \ \ N\\ \ \ H\end{matrix} \quad (VIII).$$

The aziridine ring is responsible for the reactivity of aziridines in relation to nucleophilic agents. A characteristic property of the ring is the ease of addition of a proton to the nitrogen atom, the high reactivity of the carbon atoms, and the ease with which the ring is opened to form a chain (IX):

$$\begin{matrix}H_2C-CH_2\\ \diagdown\ \diagup\\ \ \ NH\end{matrix} \xrightarrow{H^+} \begin{matrix}H_2C-CH_2\\ \diagdown\ \diagup\\ \ \ NH_2^+\end{matrix} \longrightarrow H_2N-CH_2-CH_2 \quad . \quad (IX)$$

The overall mechanism of the alkylation of a nucleophilic centre A by aziridine can be represented as follows [reaction (X)]:

$$\begin{matrix}H_2C-CH_2\\ \diagdown\ \diagup\\ \ \ NH\end{matrix} \xrightarrow{H^+} \begin{matrix}H_2C-CH_2\\ \diagdown\ \diagup\\ \ \ NH_2^+\end{matrix} \longrightarrow H_2N-CH_2-\overset{+}{C}H_2 + \bar{A} \longrightarrow H_2N-CH_2-CH_2A \quad . \quad (X)$$

Sulphur, nitrogen, and oxygen atoms, which usually form part of the genetic complex [see reactions (I), (II), and (III)] are most often alkylated in biological systems ($S > N > O$). In addition, their nucleophilic properties depend on the relative polarisability of the atom. Sulphur and nitrogen atoms, which have a high atomic weight, are more readily polarised and the compounds incorporating these elements in their molecules are powerful nucleophilic agents.

The high reactivity of alkylating compounds suggests that they can react with the majority of the substances present in each cell and containing nucleophilic groups. Such groups may be the carboxy-, phosphoryl, sulphydryl, and also amino-groups[57].

Hydroxy- and carboxy-groups of the protein complex and of aminoacids[58] and also reactive amino-groups and sulphydryl groups [see reaction (I)] should be included among biological centres capable of undergoing alkylation under mild physiological conditions (as aziridine penetrates the cell).

Carbohydrates are not as a rule alkylated, since their polar hydroxy-groups are present in an undissociated form at the physiological pH.

In contrast to glycerol esters, free aliphatic acids can be esterified on alkylation. A number of vitamins ($B_1$, $B_6$, biotin, and folic and *p*-aminobenzoic acids) each contain several centres undergoing alkylation. Such centres are nitrogen atoms in rings, and amino- or carboxy-groups. This accounts for the finding that not only the protein complex but also the prosthetic groups in enzymes may constitute centres sensitive to alkylation[18].

The study of a number of reactions of alkylating agents with highly purified native DNA and its components[59-61] and also with the transforming factor under mild physiological conditions[59,60] revealed the presence in DNA of several highly reactive nucleophilic centres.

The mole of an aziridine derivative consists of an active part (one or several aziridine rings) and a "carrier"—the residual component of the molecule, and can be represented schematically by formula (XI):

$$\begin{matrix}R_3\diagdown\\ R_2\diagup\end{matrix}C-C\begin{matrix}\diagup R_4\\ \diagdown R_5\end{matrix} \quad (XI) \quad .$$
$$\qquad\quad N$$
$$\qquad\quad |$$
$$\qquad\quad R_1$$

Among the aziridine derivatives investigated are compounds containing 1, 2, 3, 4, 6, and 8 alkylating rings. The increase of the sterilising activity of ethyleneimine derivatives is as a rule directly proportional to the number of aziridine rings. In investigations much attention is devoted to the problem of whether one or several aziridine groups are required for an effective chemosterilant[15,45,62,63]. Experimental data show that monofunctional derivatives

are relatively ineffective as chemosterilants. Nevertheless the number of aziridine groups cannot be directly linked to the activity of the compound. For example tepa (with three aziridine groups) injected into male house flies proved to be more effective than apholate, which has six aziridine groups[64]. Moreover, the difference between the activities of difunctional and trifunctional aziridines may be much less than the difference between the monofunctional and difunctional compounds[6]. Certain diaziridines proved to be more effective than triaziridines with a similar structure[63,65,66].

The higher effectiveness of polyfunctional derivatives compared with monofunctional derivatives served as a basis for the hypothesis that polyethyleneimines can link DNA molecules in pairs and thereby bind pairs of chromosomes in the metaphase[67,68].

Examination under the microscope of the sexual cells of animals sterilised by polyfunctional compounds indeed reveals bridges between chromosomes. This is the reason for incorrect division of chromosomes among the daughter cells, which leads to genetic abnormalities and the non-viability of the daughter cells.

However, this hypothesis does not account for the mechanism of the mutagenic effect of monofunctional aziridines on mammals[50], although there is no doubt about the higher activity of polyfunctional compounds.

The sterilising activity of aziridine derivatives depends not only on the number of functional groups but also on the substitution at the carbon and nitrogen atoms of the ethyleneimine ring. The substitution of one hydrogen at a carbon atom of the aziridine ring sharply lowered the sterilising activity and the substitution of two hydrogen atoms resulted in its complete loss. Numerous reports lead to the conclusion that any substitution of hydrogen at the carbon atoms in the rings of aziridine compounds leads to a decrease of their sterilising activity on insects[19,26,45,56,63,65,69,70]. At the same time methylation is usually accompanied by a sharp lowering of the toxicity of the preparation. Substitution of one hydrogen atom lowers the toxicity of aziridine homologues by a factor of twelve in relation to rats and substitution of two atoms lowers the toxicity by a factor of twenty compared with unsubstituted aziridine[57]. This property of ethyleneimine derivatives leads to the possibility of seeking compounds whose toxicity for insects and warm blooded animals is low but which are still sufficiently effective for the purpose of sterilisation.

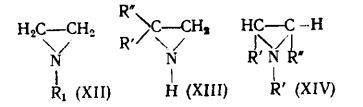
Substitution of the hydrogen atom at the nitrogen atom of the aziridine ring leads to analogous phenomena. Substitution of the hydrogen atom by aliphatic radicals lowers the toxicity of aziridine homologues in relation to rats by a factor of 10–20, substitution by aromatic groups lowers the toxicity by a factor of 40, and substitution by aliphatic-aromatic groups lowers it by a factor of 20–100 compared with unsubstituted aziridine[57]. Unfortunately, there are still insufficient data in the available literature for the analysis of the sterilising activity of such aziridine derivatives. On the other hand, acylation of aziridine (i.e. addition of an acid group to the nitrogen atom in the ring) increases the sterilising activity of the compound as well as its toxicity[28].

Moreover, the sterilising activity depends not only on the effective component but also on the nature of the "carrier component" of the molecule. For example, a decrease of the electronegativity of the central atom to which the aziridine groups are joined as a result of the replacement of oxygen by sulphur also lowers the sterilising activity of the compound. It must be emphasised that the substitution of hydrogen at a nitrogen atom in the "carrier component" of the molecule lowers primarily the toxicity, but has much less effect on the sterilising activity. In addition, some workers believe that the toxicity of chemosterilants is in general a function of the "carrier component" and is not closely linked to the number of aziridine groups in the molecule[48].

The most effective chemosterilants will probably be compounds in which aminoacids, peptides, nitrogen bases, etc. are linked to the "active component" of the molecule (aziridine ring), since natural groups apparently more readily transmit alkyl groups to cellular systems important in the vital processes of the cell and ensure their blocking. This approach has already been used in relation to compounds employed in the chemotherapy of malignant neoplasms[71].

All the aziridine derivatives already investigated can be divided (according to their chemical structure) into several groups.

C- and N-substituted derivatives of the type



(XII) (XIII) (XIV)

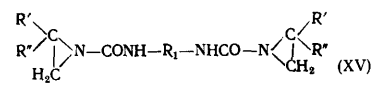
The simplest compound of this series is aziridine itself (ethyleneimine, dimethyleneimine, azacyclohexane). Experiments on the antiblastic effect of aziridines reveals its high toxicity ($LD_{100}$ = 5 mg kg$^{-1}$ for rats when administered intravenously). It has been established that the inhibition of the growth of tumours is achieved only on introduction of toxic (4 mg kg$^{-1}$) or the maximum tolerated (2.5 mg kg$^{-1}$) doses.

Aziridine and its homologues and also aliphatic derivatives in doses less than the maximum tolerated amounts stimulated the growth of tumours. Aromatic and aliphatic-aromatic compounds did not show this effect when administered in such doses, but some of them caused a weak inhibition of the growth of tumours. Aliphatic-aromatic ketones proved to be quite inactive in relation to sarcoma[57].

Unsubstituted aziridine showed the greatest mutagenic activity. A sharp reduction of mutagenic activity is observed in all cases of *C*- and *N*-substitution[72]. However, mutagenic activity cannot be fully identified with the sterilising activity of the compound, which involves a more complex process. Although chemosterilants without mutagenic activity exist, the majority of compounds sterilising insect males are as a rule mutagenic also.

Unsubstituted aziridine has been used to sterilise the Callitroga fly[56]. Compounds of this series are probably not very promising as chemosterilants, partly in view of their fairly high toxicity and partly because of their monofunctional nature.

Aziridine derivatives of urea (XV):



(XV)

It has been noted that the toxicity of compounds of this type and also their antiblastic (and probably sterilising) activity falls with increase in the number of methyl groups at the carbon atoms of the aziridine ring, which constitutes yet another proof in support of the general rule (see above).

Moreover, the toxicity of many compounds with $R_1 = (CH_2)_n$ falls as the number of methylene groups is reduced. Among derivatives of this series the following chemosterilants of the house fly have been noted:

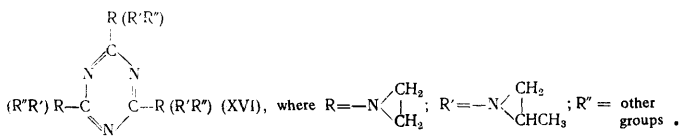
*NN'*-octamethylenebis(aziridine-1-carboxamide)[6],
*NN'*-octamethylenebis(aziridine-1-acetamide)[73],
*NN'*-heptamethylenebis(aziridine-1-carboxamide)[74],
*NN'*-pentamethylenebis(aziridine-1-carboxamide)[74],
*NN'*-phenylenebis(aziridine-1-carboxamide)[74],

and also chemosterilants of the Callitroga fly:

*NN'*-bis(aziridine-1-carboxamide)[75],
*NN'*-tetramethylenebis(aziridine-1-carboxamide)

and homologous compounds with 4–8 and 10 methylene groups[76]. However, aziridine derivatives have not found wide application as chemosterilants.

Aziridine derivatives of triazine (XVI):



(R"R') R—C C—R (R'R") (XVI), where R=—N(CH2)2(CH2); R'=—N(CH2)(CHCH3); R" = other groups.

Different derivatives may contain different combinations of the R, R', and R" groups but an obligatory condition for these compounds is the presence of at least one aziridine group.

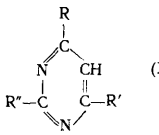
Compounds containing only one aziridine group are as a rule less toxic ($LD_{100}$ for rats is higher by a factor of 20–100 compared with aziridine and its homologues). The presence of two aziridine groups leads to an increase of the toxicity of the preparation compared with monofunctional compounds ($LD_{100}$ higher by a factor of only 1.5–6 compared with aziridine). Derivatives with three aziridine rings proved to be the most toxic ($LD_{100}$ = 0.7 mg $kg^{-1}$).[57] The presence of various substituents instead of one or two aziridine groups (alkyl, alkoxy-, halogeno-, and others) and also of substituents at the carbon atoms of the aziridine ring likewise affects the toxicity of the compound, although the effect of these [latter? (Ed. of Translation)] substituents is much weaker than the substitution of the entire ethyleneimine group.

The cytostatic activity of aziridine derivatives as well as their toxicity depends to a considerable degree on the number of aziridine rings. It is highest for triethyleneaziridines, tretamine (TET), and methyltretamine (MeTET) and decreases progressively as the number of aziridine groups in the molecule is reduced. However, a direct correlation between toxicity and cytostatic activity is not always observed. For example, 6-benzyloxy-2,4-di-(1-aziridinyl)-*s*-triazine, which has a toxicity smaller by a factor of 20–40 than that of tretamine, is not inferior to the latter in its cytostatic activity[57].

In the series of 1-aziridinyl derivatives with amino-acid residues, the most toxic are derivatives with valine and lysine residues and the least toxic are those with glycine and alanine residues and among the latter the least toxic are compounds containing the residue of the ethyl ester of $\alpha$-alanine. The introduction of an aromatic substituent into the molecule of the ethyl ester of $\alpha$-alanine results in a further decrease of toxicity by a factor of two. The cytostatic activity of these compounds is less pronounced than that of tretamine and 1-aziridinyltriazines with alkyl, alkoxy, and aryl substituents[57].

Tretamine and methyltretamine are most widely used as chemosterilants[56,77–84].

Aziridine derivatives of pyrimidine (XVII):



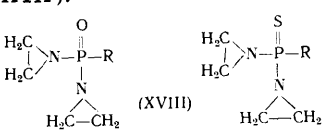
(XVII),

where R' and R" are aziridine groups and R is another substituent. The nature of the R substituent has a marked influence on the toxicity of the compounds. For example, the replacement of chlorine by a methoxy-group reduces the toxicity of the preparation by a factor greater than 6.[57]

1-Aziridinyl derivatives of pyrimidine proved to be highly active. The introduction of the minimal toxic dose leads to complete disappearance of tumour tissue from male mice[57].

These compounds are of considerable interest for the purpose of sterilisation. Several preparations have been tested as chemosterilants: (a) 2,4-di-(1-aziridinyl)-6-methyl-5-nitropyrimidine (tested on *Diptera*[85,86]); (b) 4,8-di-(1-aziridinyl)pyrimido[5,4-*d*]pyrimidine[70,74]; 1-methyl-4-methylamino-1-pyrazolo[3,4-*d*]pyrimidine[56]; (d) phosphazine[*PP*-di-(1-aziridinyl)-*N*-2-pyrimidinylphosphinic amide][76,80,87]; (e) morphimide and pyrimide‡ [*PP*-di-(1-aziridinyl-*N*-6-piperidino-4-pyrimidinyl phosphinic amide and *PP*-di-(1-aziridinyl)-*N*-6-morpholino-4-pyrimidinyl-phosphinothioic amide], which show a high activity in tests on certain insect species and only moderate activity in tests on others[88–91].

1-Aziridinylphosphine oxides (XVIII) and sulphides (XIX):



(XVIII) (XIX).

This group of compounds has attracted most attention by both physicians and entomologists. The tri-(1-aziridinyl) derivatives of phosphine oxide and sulphide (tepa and thiotepa) proved to be valuable medicinal preparations and have been successfully used in the treatment of malignant tumours and a number of chemoblastoses[92].

Di-(1-aziridinyl) derivatives of phosphine oxide with aryl substituents at the phosphorus atom are as a rule less toxic than the tri-(1-aziridinyl) derivatives (tepa). However, this cannot be explained solely by the replacement of the aziridine ring, since compounds with three and four [one and two? (Ed. of Translation)] aziridine groups proved to be no less toxic than tepa. In the first case the aziridine ring is attached to the phenyl ring via a keto-group and in the second is present as the phosphoric acid diethyleneimide residue [di-(1-aziridinyl)phosphine oxide derivative (Ed. of Translation)]. This is further proof of the effect of the "carrier component" of the molecule on the properties of aziridine derivatives§.

Compounds of this group exhibit a wide variety of cytostatic effects. In their action on malignant tumours the most active compounds approach the effect of tepa, but

‡ These are transciptions of Russian names which transliterate as morfimid and pirimid. The order of the systematic names should probably be reversed (Ed. of Translation).

§ The test in this paragraph is confused possibly owing to the omission of one or more sentences from the Russian original (Ed. of Translation).

some are much inferior and others do not show any activity at all.[57]

Di-(1-aziridinyl)phosphine oxides containing aminoacid ester residues have a low toxicity. The relations noted for the analogous derivatives of the triazine series are also shown by this group of preparations. The least toxic proved to be a compound with a glutamic ester residue ($LD_{100}$ for mice is more than ten times higher than $LD_{100}$ of tepa). Compounds of this series proved to be relatively inactive as antitumour agents. We were unable to find any data in the literature on the sterilising activity of preparations of this group.

Di-(1-aziridinylphosphine oxides and sulphides with heterocyclic substituents at the phosphorus atom are as a rule less toxic than tepa (approximately by a factor of 3–10) but exhibit a high antiblastic activity. For example, Dipin has become widely known in the chemotherapeutic treatment of cancer. Certain compounds of this group are known as highly effective chemosterilants, for example Phophazine and compounds with a pyrimidine ring (these were discussed above; see the aziridine derivatives of pyrimidine), Dipin (1,4-piperazinediylbis[di-(1-aziridinyl)]-phosphine oxide);[81,87] derivatives with furan and toluene rings were tested without special success[70,93,94].

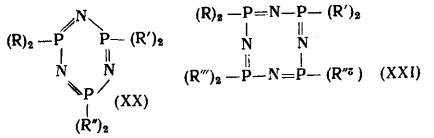
Tri-(1-aziridinyl)phosphine oxide and sulphide. As already mentioned tepa and thiotepa are most widely used in the chemotherapy of cancer. Their toxicity is fairly high but lower by a factor of 6–8 than that of unsubstituted aziridine. In entomology these compounds are known as highly active chemosterilants for a number of insects and tick species.

Tepa [aphoxide, tris-(1-aziridinyl)phosphine oxide] proved to be a highly effective chemosterilant for more than 25 insect species[10,56,69,83,86,95–114]. The methyl analogue of tepa (methaphoxide, metepa) is a preparation with a somewhat reduced effectiveness compared with tepa, but its toxicity is much lower; it was tested on more than fifteen insect species[56,69,94,98,101,103,106,109,111–123]. Tri-(1-aziridinyl)phosphine sulphide (thiotepa) is less toxic than tepa but its sterilising activity is weaker. It has also proved to be an effective chemosterilant for a large number of insect species[56,77–84,87–89,94,106,124–130]. The methyl analogue of thiotepa (methiotepa) has both a lower toxicity and a lower activity than the initial compound[56,85,97,106,131].

It is striking that the introduction into the metepa and methiotepa molecules of a further methyl group in place of hydrogen at a carbon atom of the aziridine ring leads to complete loss of sterilising activity[19,45,132].

The *PP*-di-(1-aziridinyl) derivatives of phosphinic and phosphinothioic amides and methyl phosphinothiolate are related to this group. These compounds have a low toxicity but nevertheless sterilise insects fairly actively even at low concentrations. *PP*-di-(1-aziridinyl)phosphinic amide proved to be a highly effective preparation for the codling moth[133]. *PP*-di-(1-aziridinyl)phosphinothioic amide is less active in relation to this insect[133]. Methyl di-(1-aziridinyl)phosphinothiolate is a highly active chemosterilant for the house fly[134].

Aziridine derivatives of the trimer (XX) and tetramer (XXI) of phosphonitrilic chloride:



(XX)



(XXI)

The initial compounds of this series of derivatives (the trimer and tetramer of phosphonitrilic chloride) have a low toxicity. The substitution of 4, 6, and 8 chlorine atoms by 1-aziridinyl groups leads to an increase of toxicity but it still remains of the same order of magnitude as that of tepa and thiotepa and is lower by a factor of 10–20 than the toxicity of tetramine. The introduction into the molecule of piperidine, morpholine, or glycine residues in place of chlorine atoms leads to an appreciable reduction of the toxicity of the preparations[57]. Compounds with not less than four 1-aziridinyl groups exhibit a high cytostatic activity. Their reduction to two or complete replacement of 1-aziridinyl groups fully eliminates the antiblastic activity.

Several compounds of this series have been tested as insect chemosterilants: LS-152 (monomorpholinotetra-(1-aziridinyl)chlorotriphosphonitrile)[89], methylapholate[56], and apholate. The last compound proved to be highly effective and is most widely used[9,10,48,56,69,77,78,81,82,84,85,87,91,94,96,98,100,102–105,107,109,111–114,119,121,123,126,135–143]. It showed a high sterilising activity on almost 40 species of insects and ticks[21].

## III. ANTIMETABOLITES

Antimetabolites are structural analogues of metabolites, i.e. substances necessary for the existence and vital activity of organisms in consequence of their decisive role in metabolic reactions (for example, vitamins, hormones, aminoacids, etc.). However, structural analogy with natural metabolites is not the only characteristic of antimetabolites.

Another important requirement which must be met by antimetabolites is metabolic antagonism[6]. Antimetabolites are interesting also because they can give rise in organisms (or in individual biological systems) to an insufficiency of the metabolite to which they are structurally related and the biological effect observed under these conditions can be removed in almost all cases or reduced by a simple increase of the concentration (dose) of the metabolite.

By virtue of the steric analogy, the antimetabolite competes with the metabolite and, participating together with the latter in biochemical reactions, displaces it from metabolic processes. The cell having employed antimetabolites cannot biosynthesise normal compounds (proteins, nucleic acids, and others).

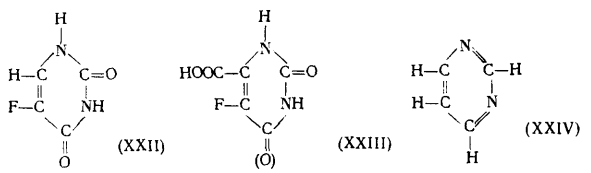
Yet another characteristic of antimetabolites is their capacity to act on females alone, particularly when administered to adult insects. The synthesis of nucleic acids in adult females is usually a prolonged process: after the emergence from the pupa, the ovaries are as a rule only partially developed, but even after the attainment of sexual maturity and mating the production of eggs continues throughout the life of the female. Since the gonads are the principal site of mitotic activity in adult insects, their specific interaction with the antimetabolite is facilitated. Immediately after the emergence from the pupa, the male has as a rule a complete set of mature spermatozoa where the formation of nucleic acids has already been completed and therefore they are more resistant to the action of antimetabolites. However, it has not been determined exactly whether antimetabolites can arrest the spermatogenesis in the males belonging to species where this process is also prolonged[19,144]. The minimum sterilising dose of the antimetabolite is low and usually smaller by a factor of 100 than that of aziridine derivatives. The toxicity of these compounds is as a rule very low and their antiblastic activity is fairly high. Moreover, it is noteworthy that

the vast majority of antimetabolites are active only when administered via the intestine and only exceptionally certain preparations exhibit contact activity.

### Analogues of Purine and Pyrimidine

Compounds of this series can act upon the normal cell metabolism in three ways: they can compete with a normal nucleotides for enzymes and thus prevent the regular combination of the nucleotides with nucleic acids, they can control by a feedback effect the synthesis of new nucleotides and hence nucleic acids, and finally they can be incorporated directly into newly formed nucleic acids and the latter, by losing their functional purpose, can give rise to pronounced metabolic disorders [19].

Although many analogues of purine and pyrimidine (XXIV) have already been tested, a detailed study has been made of only a few of them: 5-fluorouracil (XXII) and 5-fluoro-orotic acid (XXIII):



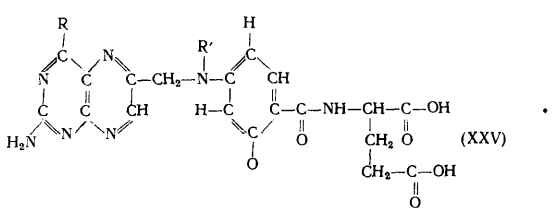

They were tested mainly on flies and mosquitoes. These compounds sterilise only the female insects [145]. 5-Fluorouracil replaces almost 50% of uracil in bacterial RNA [136] and 28–48% in TMV RNA [146] and stops their reproduction. 6-Methyluracil behaves similarly. A direct correlation has been observed between the content of 5-fluorouracil in the RNA in the eggs of the house fly and the loss of their viability [136]. It is noteworthy that in all cases the sterility was temporary and after 3–5 days was gradually eliminated [116]. 5-Fluorouracil is known as a sterilant for more than ten insect species.

5-Fluoro-orotic acid administered via the intestine proved to be effective [56,147] and the sterility was more stable—the treated females did not lay eggs. Moreover, 5-fluoro-orotic acid sterilised also males [14].

4-Amino-1-*H*-pyrazolo[3,4-*a*]pyrimidinium sulphate, which is a purine antagonist, is effective only at high concentrations [43].

### Analogues of Folic Acid

Compounds of this series have a wide variety of structures and properties. More than 50 derivatives of *p*-aminobenzenesulphonamide and other sulphonamides have been tested as chemosterilants without much success [19], but the compounds bear only a very remote relation to the structure of folic acid [6] (XXV, R = OH, R′ = H):




On the other hand, antagonists of folic acid such as aminopterine (XXV, R = $NH_2$, R′ = H) and methotrexate (XXV, R = $NH_2$, R′ = $CH_3$) are highly acid sterilants for the females of numerous insect species [6,19,38,67,131,148]. It is noteworthy that the sterility caused by methotrexate is more stable—the ovaries remain undeveloped and the females do not lay eggs throughout their life [19]. Aminopterine and its sodium salt have a similar but weaker effect [10,42,148]. It is striking that the sterilising activity of these compounds can be inhibited by the introduction of an excess of folic acid [140,148].

It has been shown experimentally that aminopterin is a radiomimetic poison; it reduces the number of mitoses. Folic acid antagonists strongly inhibit the enzymes involved in the formation of folic acid and act on the synthesis of nucleic acids by inhibiting the conversion of folic acid into folinic acid and by interfering with the assimilation of the latter in the process of synthesis [26], impair cell division, and damage chromosomes [149].

### Other Antimetabolites

Tests on numerous analogues of aminoacids, carbohydrates, steroids, and also aliphatic acids and hydroquinone derivatives showed that most of them are not very promising. Derivatives of hydroquinone [150], which have a low toxicity for insects and warm blooded animals, deserve mention. Among dimethylhydroquinones, 2,6-dimethylhydroquinone is the least toxic isomer. When tested, it proved to be a highly active chemosterilant for the females and males of the house fly [93].

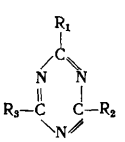
The addition of cyclopropene aliphatic acids and also of sterculic and malvalic acids to the feed lowers the egg laying capacity of chickens and the fertility of house fly females. A valuable property of these acids is their extremely low toxicity, which led some investigators [151] to suggest their inclusion as additives with certain toxic chemosterilants.

## IV. COMPOUNDS OF MIXED TYPE

In recent years an increasing number of chemosterilants have appeared which are neither alkylating agents nor antimetabolite analogues. Such compounds sometimes have a structural analogy with certain of the previously described chemosterilants, but this feature alone cannot serve as a basis for their inclusion in the corresponding groups of preparations. Although the mechanism of the action of compounds of this type has as yet been little investigated, the available data suggest the existence of fundamental differences, specific for each individual group. In this section we shall consider only certain groups of the chemosterilants.

### *s*-Triazines

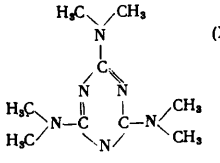
Derivatives of *s*-triazine (XXVI, $R_1$, $R_2$, $R_3$ = H) are not alkylating agents:




The substitution of any of the three hydrogen atoms in *s*-triazine by an alkyl group leads to the formation of alkyl

derivatives of triazine, some of which are well known chemosterilants (see the 1-aziridinyl derivatives of triazine) and mutagenic and antiblastic agents.

Historically, the first non-alkylating *s*-triazine chemosterilants are hemel (XXVII), hexamethylmelamine (HMM), and tri-(2,4,6-dimethylamino)-*s*-triazine [XXVII, $R_1$, $R_2$, $R_3 = N(CH_3)_2$],[66] which is a structural analogue of tretamine (TET):



(XXVII)

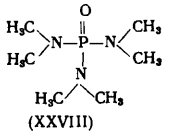
In view of the absence of aziridine rings, this compound has no alkylating properties, but it has retained the capacity to sterilise insects[66]. There exists the view that hemel is converted in animal organisms into effective chemosterilants (possibly into alkylating substances). For example, demethylation of hemel leads to the formation of formaldehyde[66]. Hemel is fairly rapidly eliminated from the insect's organism in the form of metabolities: about 50% after 5 h and about 98% after 18 h.[152]

In the last few years more than 800 derivatives of *s*-triazine were tested. Methyl-substituted melamines [XXVI, $R_1$, $R_2$, $R_3 = N(CH_3)_2$] proved to be most active chemosterilants. In this sense hemel (XXVII) may be regarded as hexasubstituted melamine. The activity of the compound increases with the number of methyl groups[131]. The substitution of one of two hydrogen atoms in the methyl groups of even penta- and hexa-methyl-substituted and *N*-propyl- and butyl-melamines leads to the loss of sterilising activity, while on their substitution by ethyl or isopropyl groups a high activity is retained by the compound. That this rule is not restricted solely to alkyl substituents, is shown by the high activity of monoacetylmelamine (XXVI, $R_1$, $R_2 = NH_2$, $R_3 = NHCOCH_3$), while the 2-hydroxyethyl derivative (XXVI, $R_1$, $R_2 = NH_2$, $R_3 = NHCH_2.CH_2OH$) and the 2-aminoethyl derivative (XXVI, $R_1$, $R_2 = NH_2$, $R_3 = NHCH_2CH_2NH_2$) proved to be inactive. The sterilising activity of mono-, di-, and tri-substituted derivatives is inversely proportional to the size of the substituting group. However, this rule does not apply to cyclic substituents[6,153]. Diamino-*s*-triazines with halogeno-, hydroxy-, alkoxy-, sulphydryl, and aryl groups show only a weak sterilising activity or none at all.

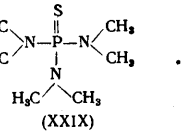
A characteristic feature of *s*-triazine derivatives is the specificity of their action: certain compounds, in particular hemel, are more effective on males than on females[62,63,66,70], but the majority of others are sterilants only in relation to females. Moreover, some of the compounds exhibit species specificity: for example, 2,4-diamino-6-(2-furyl)-*s*-triazine (XXVI, $R_1$, $R_2 = NH_2$, $R_3 = C_3H_3CO$) is a highly active chemosterilant of the house fly but does not have a significant influence on mosquitoes, the Mexican fruit fly, the Callitroga fly, and many other insects[6]. Possibly this compound will prove to be the first of a series of specific sexual sterilants which will be synthesised in the future for other insect species as well.

## Phosphoramide Derivatives

Among these compounds, mention should be made in the first place of hexamethylphosphoramide [HMPA, hempa, tris(dimethylamino)phosphine oxide] (XXVIII), which may be regarded as a structural analogue of tepa:



(XXVIII)



(XXIX)

Hexamethylphosphoramide is the first highly effective chemosterilant in the series of numerous similar compounds which have been tested[13,40,63,66,88,89,154,155]. The majority of its derivatives are inactive and only some of them showed moderate sterilising activity.

Phosphorothioic hexamethyltriamide [thiohempa, tris-(dimethylamino)phosphine sulphide] (XXIX) may be regarded as a structural analogue of thiotepa. In its sterilising activity on certain insect species, it approaches hexamethylphosphoramide[6,155,156].

The substitution of larger alkyl or aryl groups or heterocyclic groups for hydrogen at a carbon atom leads to a lowering or loss of sterilising activity by compounds of this series.

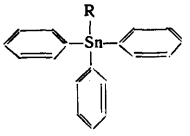
The activity of hexamethylphosphoramide in relation to certain insect species is very variable. The initial success is sometimes not repeated in subsequent tests (or in the experiments of some investigators hexamethylphosphoramide proved to be inactive in relation to insect species for which it had been claimed to be highly active in other experiments). The reason for this conflicting nature of the results is not as yet clear.

Although hexamethylphosphoramide and phosphorothioic hexamethyltriamide do not exhibit alkylating properties, their well defined physiological and cytotoxic properties give rise to changes in the cell, which resemble the phenomena occurring under the influence of alkylating agents[157]. This is consistent with the hypothesis that, like hemel, hexamethylphosphoramide is demethylated in the insect organism (in particular in the house fly) with formation of an effective alkylating agent—formaldehyde[66]. However, the latest studies[158] do not confirm these data.

The structural similarity of these compounds to aziridine derivatives and the relative simplicity of their molecules make them extremely valuable for the elucidation of the mechanism of sexual sterilisation.

## Triphenyltin Derivatives

Preliminary tests on several trialkyl- and triphenyl-tin derivatives (previously employed as fungicides and insecticides) in 1960–1962 showed that they are either highly toxic or exhibit a moderate sterilising activity. After 1964 a series of studies were made on compounds of this type[74,113,159,160] exhibiting sterilising activity in very low doses. High activity is shown only by triphenyltin derivatives (XXX, R = halogen, hydroxyl, alkyl, ether group, etc.) but certain other derivatives can also show a limited activity:



(XXX)

The properties of triphenyltin derivatives indicate an activity of the antimetabolite type. They are active exclusively on insect females and the sterility which they

cause is as a rule temporary. Moreover, they are effective only when administered via the intestine. Triphenyltin derivatives are highly toxic, which may be an obstacle to the sterilisation of many insect species.

### Other Agents of the Mixed Type

The rapid growth of the range of substances tested as insect chemosterilants led to the discovery of sterilising activity in many compounds. Some of these deserve at least a brief mention.

In the last few years more than 100 derivatives of urea and thiourea were tested. Urea and thiourea show variable results[84,141,142,148], which can be accounted for by their toxicity for insects. The most active are cyclic derivatives of urea and thiourea[139], but they too act only on the females of certain species. Urea derivatives containing alkylating groups and known as cytostatic agents in the chemotherapy of cancer and also as effective chemosterilants were discussed above (see the 1-aziridinyl derivatives of urea).

To so called "natural products" constitute another category of compounds[6]. In the first place mention should be made of pyrrolizidine alkaloids[161,162]. However, their effectiveness is extremely limited: among the species tested, only the males of the house fly proved to be susceptible to monocrotaline. The products of the hydrolytic decomposition of monocrotaline did not exhibit a sterilising activity.

Hydroquinone derivatives were discussed above as antagonists of progesterone derivatives and will not be considered further here. Certain antibiotics should also be mentioned (streptomycin, biotin, and others)[163,164].

Colchicine was described as one of the first chemosterilants of Drosophila females as early as 1947.[165] Unfortunately its toxicity for many species and its specific effect of females alone greatly reduces the scope of its applications[85,94,166].

Compounds capable of acting selectively on the hormonal control of reproductive functions are of considerable interest. Here undesirable effect on other functions of the organism can be greatly limited. Only the first step has been taken on these lines[38,39,167-170], but they open up vast prospects for this method of influencing the populations of harmful species.

Many organic and inorganic insecticides[37,38,41,43,171-173] in sublethal doses suppress to a greater or less extent the reproduction of insects. These compounds are probably not of great interest as sterilising agents because the sterility in such cases may be a consequence of the general debilitating effect of the sublethal doses. On the other hand, cases of an increase in the fertility of insects under the influence of insecticides are well known. However, any effect of insecticides, inhibiting or stimulating reproduction, may be important in the assessment of the long term consequences of their application.

Recent studies led to the discovery of sterilising activity in certain groups of compounds which were neither mutagenic agents, nor antimetabolities, nor insecticides. For example, various organic and inorganic boron compounds proved to be chemosterilants for *Cochliomyia hominivorax* Coq. Here the sterilising activity is directly proportional to the amount of boric acid produced in the hydrolysis of the test compound[174].

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In conclusion it is necessary to consider certain requirements which must be met by compounds tested as chemosterilants. High sterilising activity depends to a considerable degree on the chemical nature of the compounds, on the steric positions of its atoms, and also on the physical properties (solubility, capacity for migration, and others). A low solubility of the preparation gives rise to difficulties not only in the technique of its application, but also to a considerable degree determines its capacity for migration within the organism.

The toxicity of the compound for insects, warm-blooded animals, and man is also an important criterion of its usefulness as a chemosterilant. Preparations which are highly toxic for insects can appreciably lower their viability apart from sterilisation (reduction of the life-span of the treated individuals, lowering of the competitive capacity of males or the competitive capacity of spermatozoa, etc.). This can appreciably reduce the effectiveness of the method of sexual sterilisation. It should be noted that all compounds with sterilising activity are more or less toxic for insects. A criterion of the effectiveness of the preparation may be the ratio $LD_{50}/ED_{50}$ or $LD_{100}/ED_{100}$, which has come to be called the "safety factor", where $LD_{50}$ and $LD_{100}$ are lethal doses for 50% and 100% of the individuals and $ED_{50}$ and $ED_{100}$ are the corresponding doses causing sterility; the chemosterilant may be regarded as more effective the higher is the ratio. A "safety factor" ($SF_1$) of 5 and above is regarded as satisfactory for the majority of chemosterilants[26]. However, it is not constant for all values of $LD$ and $ED$ and therefore, for a complete characterisation of the sterilant, it is necessary to determine a further two safety factors ($SF_2$ and $SF_3$).[65,66,140] The "safety factor" $SF_2$ may be defined by the formula

$$SF_2 = \frac{LD_{0.01} - ED_{99.99}}{ED_{99.99}} .$$

The third "safety factor" $SF_3$ is similar to $SF_2$:

$$SF_3 = \frac{LD_{0.01}}{ED_{99.99}},$$

where $LD_{0.01}$ is the maximum dose tolerated (or the dose resulting in the death of 0.01% of the individuals) and $ED_{9.99}$ is the minimum dose, sterilising 99.99% of the individuals. Compounds for which $SF_2$ is equal to or greater than zero and $SF_3$ is equal to or greater than unity can sterilise all insects without killing them. Preparations with negative $SF_2$ and $SF_3$ greater than $-1$ may be used to sterilise insects but then some of the treated individuals are destroyed. The degree of risk for man and warm-blooded animals is various for different groups of chemosterilants. The most dangerous and toxic are the derivatives of chloroethylamine and aziridine, which prevents their large scale practical application.

The phytotoxicity of sexual sterilants has been less investigated. Only apholate is an exception in this respect. Plants tolerate spraying with 1–2.5% solutions of the preparation without harm and only spraying six times in succession moderately retards the growth of plants, causes the dying off of leaves etc.[22] Naturally, even moderate phytotoxicity can limit the application of the chemosterilant in the treatment of populations under natural conditions.

The stability of the compounds can also be of great importance. Aziridine derivatives readily polymerise, are unstable in an acid medium, but are usually stable in an alkaline medium. The instability of the preparations makes their application difficult. The volatility of the compounds is also undesirable since it increases the

danger from the preparation and can give rise to additional technical difficulties. On the other hand, it eliminates the problem of stable residues of chemosterilants. The high rate of metabolism of chemosterilants in warm-blooded animals and man, which, however, depends on the reactivity of the compound, somewhat reduces the potential danger of these substances.

The universality of the action of chemosterilants has a marked influence also on their effectiveness. The most effective preparations are active when administered both via the intestine and on contact. When the preparation administered in a particular way is inactive, it gives rise in the first place to technical difficulties in its application, but its effectiveness is only indirectly influenced. However, some chemosterilants are capable of sterilising only members of one sex. This property is directly reflected in their effectiveness. A preparation having a 95% activity and sterilising both males and females is twenty times more effective than a preparation capable of sterilising only members of one sex[12]. Moreover, the separations of the released insects in accordance with their sex is not always technically feasible.

Another requirement which must be met by chemosterilants is specificity of their action. The majority of the existing sterilants act not only on insect species but also on other invertebrate and warm-blooded animals. When sterilisation procedures based on the treatment of the pest population under natural conditions are employed, the specificity of the method is ultimately determined by the specificity of the action of the chemosterilant employed. Naturally, compounds exhibiting not only group specificity (i.e. acting on representatives of the same order, family, and genus) but also species specificity are necessary. However, difficulties then arise in the determination of the sterilising activity of the preparation, since tests are carried out a limited number of objects (the house fly, meat flies, etc.). When sexual sterilisation involving the release of sterile insects is employed, the problem of the specificity of the action of the preparations is not quite so acute, but this procedure is more laborious and expensive, since it necessitates the preliminary mass breeding of an enormous number of insects.

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