Science Dossier

How chlorine in molecules affects biological activity

Klaus Naumann, Bayer CropScience

November 2003

November 2003

This publication is the fourth in a series of Science Dossiers aiming to provide the scientific community with reliable information on selected issues.
If you require more copies, please send an email indicating name and mailing address to eurochlor@cefic.be.

The document is also available as a PDF file on www.eurochlor.org

Science Dossiers published in this series:

1. Trichloroacetic acid in the environment (March 2002)
2. Chloroform in the environment: Occurrence, sources, sinks and effects (May 2002)
3. Dioxins and furans in the environment (January 2003)

Contents

Foreword

The Monitoring & Environmental Chemistry Working group (MECW) is a science group of Euro Chlor, which represents the European chlor-alkali industry. The main objectives of the group are to identify both natural and anthropogenic sources of chlorinated substances, study their fate, gather information on the mechanisms of formation and degradation in the environment, and achieve a better knowledge of the persistence of such substances. The MECW often uses external specialists to assist in developing reports that review the state of existing knowledge of the different aspects mentioned. The principal investigator collects information from the scientific literature and available data regarding natural or anthropogenic emissions in the environment, with the objective to cover all the aspects described above.

Dr Klaus Naumann worked in pesticide research at Bayer's principal scientific laboratory in Leverkusen from 1969 until his retirement in early 2003. Between 1979 and 1989 he led chemical research into insecticides for Bayer's crop protection division. His main areas of expertise are organic synthesis in phosphorus chemistry and pyrethroid insecticides. He has also worked in heterocyclic and aliphatic synthetic chemistry, natural compounds (including naturally-occurring organochlorine compounds) and the mode of actions of biologically-active compounds. One of his main achievements was his involvement in the discovery of a new lead structure. Since 1990 he has been head of international research co-operation as well as manager of pesticide research. Dr Naumann has been a member of Euro Chlor's MECW since 1996. Before joining Euro Chlor he was awarded the American Chemical Society's (ACS) International Award for Pesticide research. His interest in the politics of communication between industry and the general public has prompted his involvement with providing objective, scientific information for ACS.

Over the years there has been much debate in the media, among environmentalists and politicians about chlorine chemistry and in particular persistent polychlorinated chemicals, used for many years but no longer manufactured or marketed. Further experiments are still needed to establish how chorine influences the biological activity of a potential drug or crop protection agent. Investigations into the several hundred chlorinated compounds registered by the authorities as pharmaceutical drugs or crop protection agents show that it is not true to say that all chlorinated chemicals are dangerous. The chlorine atom is a modulator of activity and it has been proved that the introduction of a chlorine atom into specific positions of a molecule may improve biological activity. Chlorinated organic chemicals in the molecular weight range between 200 and 600 constitute an indispensable segment in the range of existing biologically-active chemicals used as pharmaceuticals or crop protection agents. There is no doubt that the chlorine atom will remain an important tool for probing structure-activity relationships in life science research and as a molecular component in commercialised compounds.

Summary

A number of well-known polychlorinated chemicals are toxicologically and environmentally unsafe. Opponents of chlorine chemistry often target these substances because of their persistence. However, chlorinated organic chemicals in the molecular weight range between 200 and 600 constitute an important and indispensable segment in the range of existing biologically-active chemicals used as pharmaceuticals or crop protection agents.

Over time it has been found empirically that the introduction of a chlorine atom into one or more specific positions of a biologically-active molecule may substantially improve the intrinsic biological activity. In some cases the presence of a chlorine atom is crucial for the activity of a compound derived from nature or by chemical synthesis such as the diverse compounds **1** to **12** and **23** to **30** (see Scheme 9 and Table 3). But in other cases chlorination diminishes or abolishes biological activity as shown for the chlordane homologues **139** to **143**.

Thus a chlorine atom, like any other substituent, is a modulator of activity as represented in the many examples **31** to **124** (see tables 4-7). Almost all non-reactive chlorinated and chlorine-free chemicals are devoid of any biological activity at the highest concentration typically used in primary screening tests employed to discover useful biological properties.

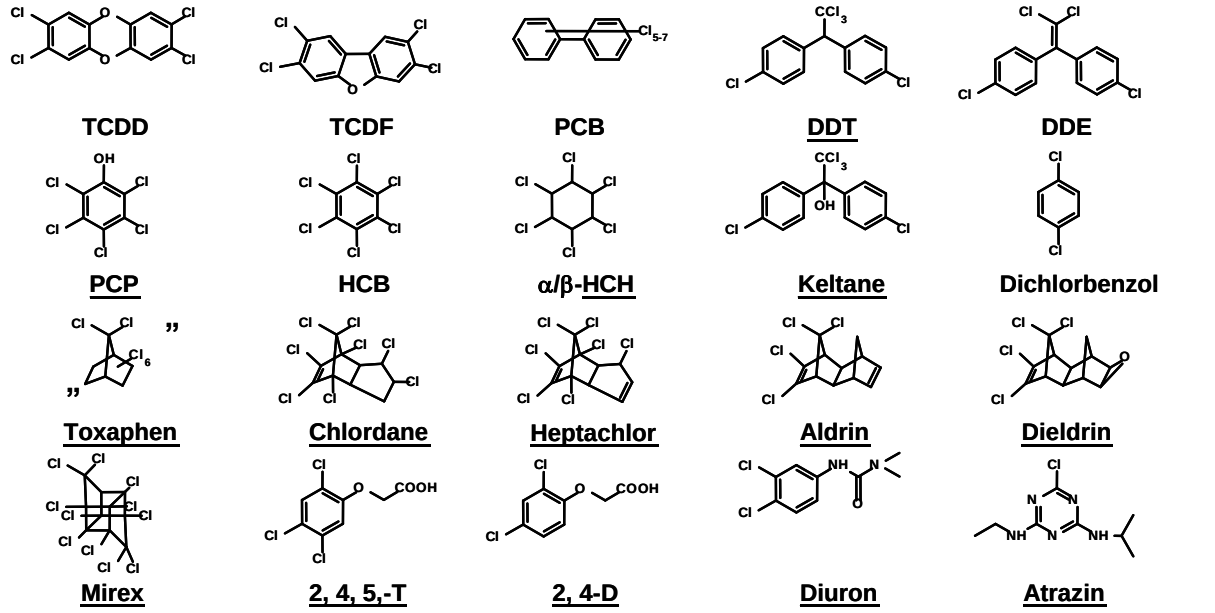
The influence of a substituent such as chlorine on the biological activity of a potential drug or crop protection agent still has to be established empirically in biological experiments designed to detect desired activity or toxicological properties. Sometimes chlorine does prove to be the optimum substituent of a chemical for improved activity.

Long-term rigorous investigations of several hundred chlorinated compounds, registered by the authorities as pharmaceutical drugs or crop protection agents, show that the generalisation “all chlorinated chemicals as a rule are dangerous” is not justified. This can be deduced from the negative toxicological properties of a hundred chlorinated and reactive compounds of low molecular weight that are relevant in terms of safe working conditions in the chemical industry and for ecological safety.

Chlorinated compounds are not generally toxic or dangerous. Highly reactive chemicals or polychlorinated compounds cannot be compared with regard to toxicological properties with unreactive compounds having a low degree of chlorination. The chlorine atom, as one of many possible substituents used in synthetic organic chemistry, will remain an important tool for probing structure-activity relationships in life science research and as a molecular component in commercialised compounds.

1. Background[1)]

Chlorine is often in the public spotlight, mostly because of concerns about products—such as PCBs—used for many years, but now no longer manufactured or marketed. The ecological persistence, negative biological effects and/or other unforeseen negative properties of some organochlorine chemicals (Scheme 1), are the reason for discussion in recent years at a high political level about a ban [1] on chlorine and on all chlorinated organic chemicals.



Scheme 1 - Organochlorines in the public debate

The possibility of carcinogenic and estrogenic effects is of paramount concern. Organochlorines[2)] account for about 15% of the official list of organic chemicals that require special safety precautions to be taken in the workplace. Some 13%, or 1,050, of all existing chemicals traded in amounts larger than 1,000 tonnes/year are chlorinated organic compounds. Seventy-three of these are intermediates, not intended for administration to humans or release into the environment.

The specific toxic properties of about 120 thoroughly investigated volatile chemicals, containing one to six carbon atoms and with a greater or lesser degree of chlorination, have been extensively discussed recently [2, 3]. Most of these are used as solvents or chemical reagents. From this data a general rule was deduced that introduction of the chlorine atom(s) into the parent molecule increases toxicity and leads to carcinogenic and mutagenic properties.

About 45% of plant protection agents marketed since 1989, 13 % of current pharmaceutical drugs, and many technical materials containing a chlorine-carbon bond are more effective in terms of performance and cost/benefit ratio than their chlorine-free parent molecules.

Industrial chemists have always tried to optimise these two aspects. One of the various approaches is to test whether chlorine in a given case would improve the desired beneficial properties or not. In retrospect one must admit that the risk to humans and nature was sometimes neglected—certainly during the early days. Often this was

1) Based on the paper “Influence of Chlorine Substituents on Biological Activity of Chemicals” published in J. Prakt. Chem. **1999**, 341, 417 – 435 and Pest. Manag. Sci. 56; 3 – 21 (2000)

2) Even this term is disputed. In some circles the term “organochlorine” is strictly confined to ecologically troublesome polychlorinated chemicals. It is used here as chemists in all countries do, in accordance with custom in chemical science and the corresponding publications media, by creating the organo-element term, as in organophosphorous-, organotin-, organosulfur- etc. It simply means a chemical compound in which a chlorine atom is bound covalently to a carbon atom, forming a C-Cl bond.

due to naïve assumptions or an insufficiently advanced state of the science involved. One should consider, for example, the tremendous advances in trace analysis methodology over the past three decades.

As a rule it is only scientific success stories that are published in journals and patents. As a result information contained in the scientific literature is biased towards the effects of chlorine on the properties of compounds synthesised for biological activity. There have been repeated complaints by university scientists that industry—as the only place where generalised data on the structure/activity effects of chlorine substituents on biological properties are gathered in the course of its research—is hiding such data.

It should be appreciated that industrial scientists are not motivated to prepare results for scientific publication and peer review if they have no practical relevance or are not interesting. In this paper some of this data, though not very polished, is presented. It is impossible for such information to be comprehensive. Some is considered as intellectual property. Much information is unpublished because it is only raw screening data. Nevertheless, in principle it can be repeated experimentally and thus corroborated.

In view of ongoing discussions outside the scientific community directed specifically against the use of chlorine, this article concentrates on the influence of the element on the biological activity of chemicals.

However, chlorine is just one substituent that can influence the properties of substances. Each substituent may have its particular merits in any given case of a biologically-active molecule. Industrial chemists are generally pragmatic and have no reason for using chlorine when it is not justified.

2. Biological Activity

There is a typical “biological activity” for each chemical; it comprises any effect caused in a biological object, not only toxicity. It depends on the chemical structure. Acute toxicity can be compared in terms of the oral dose that kills 50 % of a cohort of test animals (LD_{50}), usually rats. Table 1 shows that the LD_{50} can extend over a vast dose range of more than 10 or more orders of magnitude. The LD_{50} can be influenced by factors up to 10 depending on the medium in which the chemical is bioavailable, for example solvent, oil or detergent. The acute toxicity of a given chemical may also differ by some orders of magnitude amongst related animal species, as was found for TCDD (Table 1).

These phenomena are the basis for selective herbicides, which may eliminate weeds (eg wild oat weeds in an oat field). Very high toxicity against weeds is not necessarily correlated with toxicity to warm blooded animals. In the course of tests prior to registration the correlation turned out to be true only for a very few cases. Moreover, any kind of toxicity is a matter of applied dose. This basic law of pharmacology and toxicology was discovered by Paracelsus 450 years ago. He ruled: “dosis facit venenum”. It is the basis for using certain toxic compounds at low non-toxic doses as medicines. Vice versa, everything at high doses can be toxic, like table salt.

In addition to the structure and dose of a chemical, the time frame of action determines its overall effect on a biological object such as an animal, plant, micro-organism, insect, human or even a whole biocenosis, see Table 2.

Table 1 - Scope of acute toxicity of substances

Substance	Chlorine content	Origin	Minimal Lethal Dose LD50 µ/kg p.o. rat	
Botulinum toxin	-	bacterium	0.00003	
Tetanus toxin	-	bacterium	0.0001	
2, 3, 6, 7-Tetrachlorodibenzodioxin	+	Synthetic + natural chemical	1	guinea pig
TCDD			45	rat
			115	hare
			5.000	hamster
Saxitoxin	-	Fish	9	
Bufotoxin	-	Toad	390	
Curare	-	Plant	500	
Sarin	-	Synthetic warfare chemical	550	
Muscarin	-	Toadstool	1.100	
NaCN	-	Chemical	10.000	
Parathion E 605	-	Synthetic insecticide	13.000	
Dieldrin	+	Synthetic insecticide	46.000	
Pentachlorophenol	+	Synthetic fungicide	50.000 – 500.000	
Chlorpyrifos	+	Synthetic insecticide	96.000 – 270.000	
DDT	+	Synthetic insecticide	113.000	
Lindane	+	Synthetic insecticide	120.000	
Caffeine	-	Plant	170.000	
Nicotine	-	Plant	170.000 – 350.000	
Trichlorfon	+	Synthetic insecticide	225.000	
Permethrin	+	Synthetic insecticide	430.000 – 4.000.000	
4-Chlorophenol	+	Synthetic chemical	670.000	
2, 4 D	+	Synthetic herbicide	700.000	
2, 4, 5-Trichlorophenol	+	Synthetic chemical	820.000	
Atrazine	+	Synthetic herbicide	1.780.000	
Diuron	+	Synthetic herbicide	> 5.000.000	

Some effects are very short lasting, reversible or irreversible, caused by short-term exposure to a natural or synthetic chemical, for example odour, hormonal effects or acute poisoning. Others are intended to last longer, such as some drugs and pesticides. Pharmaceuticals regulate biochemical-physiological processes in humans, while pesticides regulate processes in order to kill a weed, a caterpillar, fungus or bacterium. Some long-term effects are

undesired, as in the case of environmental pollution or worker exposure. A toxicological evaluation of a chemical includes the application of high doses to species over a long time span—that is chronic toxicity testing.

Table 2 - Biological activity of substances as a function of dose and duration of action

Kind of action	Dose [a] of Substance (mg) per 70 kg of a Person/Animal or per Square Meter [c]	Duration of Action [b] (Seconds)	Timescale
Signalling compound (smell, taste)	$10^{-3} – 10^{1}$ = 0.001 –10 mg	$10^{0} – 10^{1}$	Seconds
Hormones	$10^{-2} – 10^{1}$ = 0.001 – 10 mg	$10^{1} – 10^{5}$	Seconds – minutes – days
Acute intoxication	$10^{0} – 10^{4}$ = 0.1 mg – 10 g for the scope of data (see Tab. 2)	$10^{1} – 10^{2}$	Seconds - minutes
Narcotic drugs	$10^{1} – 10^{5}$ = 10 mg – 100 mg	10^{4}	Hours
Pharmaceuticals	$10^{0} – 10^{2}$ = 1 mg – 100 mg – 1 g	$10^{3} – 10^{5}$	Minutes – hours – 1 day
Accidents	$10^{3} – 10^{5}$ = 1 g – 100 g	$10^{0} – 10^{2}$	Seconds – minutes
Bad working conditions	$10^{1} – 10^{3}$ = 1 – 10 mg – 1 g	$10^{5} – 10^{7}$	Hours – days – months
Toxicological studies	$10^{4} – 10^{5}$ = 10 g – 100 g	10^{7}	One month – 24 months
Pesticides	$10^{0} – 10^{2}$ = 1 mg – 100 mg	$10^{4} – 10^{6}$	Days – week
Longlasting environmental pollution	$10^{2} – 10^{4}$ = 100 mg – 10 g	10^{7} - 10^{9}	Years - decades

a) Dose: scale of variation on mg base: nine orders of magnitude
b) Time scale of variation on a second base: nine orders of magnitude
c) Area treated/contaminated with a chemical

In Table 2 the various situations for biological effects caused by chemicals are shown in relation to the amount of the compounds and time frame of interaction with a biological system. It is evident from this scheme that the term “biological activity” is very complex and always needs more precise additional comments as to the kind of experiment or event, dose and biological object.

In this review we consider a biological effect caused by a physiological relevant dose of a substance that is not more than 1,000 times greater than the intended commercial administration rate. This is the usual upper limit for the primary testing of a chemical for discovery of intrinsic biological properties in an industrial screening programme. For agrochemicals, this means an application rate of less than 5,000 ppm or 5 kg/ha; in pharmaceutical terms a dose less than 10,000 mg/kg body weight or 10^{-4} molar. Higher doses occur only in an accident during manufacture or transport or in cases of deliberate misuse, for example suicide.

Under reasonable restrictions, the vast majority of chemicals investigated in standardised industrial screening assays are biologically *inactive* in the given *in-vitro* or *in-vivo* test even at the highest concentrations. In an attempt to beat these statistical odds, high throughput and combinatorial chemical technology have been developed over the past 10 years. Normally, such chemicals are administered only once in the screening test. Repeated administration, which would detect any possible delayed or cumulative toxic effect, is not carried out unless a compound is a candidate for development.

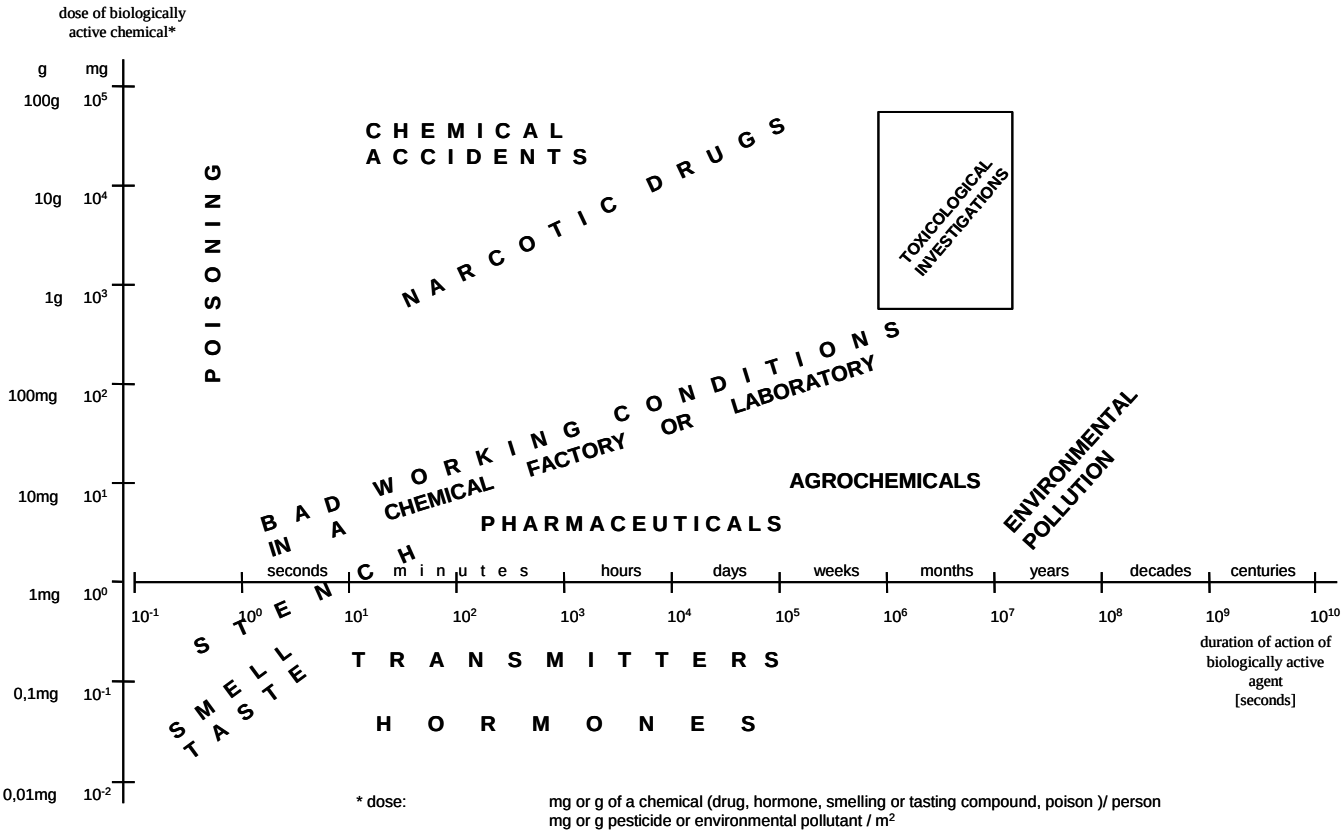
In such cases (the hope of every chemist and biologist involved) the corresponding toxicological data have to be generated in long-term standardised studies according to official guidelines. Under the current state of the art it is necessary to prove the safety of a chemical and demonstrate lack of any relevant risk by *in vivo* experiments. Many development candidates are lost during this process.

Such chronic toxicological studies on experimental animals at the maximum tolerated doses have been carried out over the last 50 years in many companies on about 1,000 agrochemicals and 3,000 pharmaceuticals, of which about 16% were chlorinated.

3. Origin of Biological Activity of Chemicals

3.1. Chemical reactivity of the carbon-chlorine bond as cause of interference of chemicals with biological systems

The properties of the carbon-chlorine bond (C-Cl) in organochlorines have been analysed by Henschler [2, 3]. In the low molecular weight chemicals investigated, the electrophilic reactivity of the carbon centre adjacent to the chlorine atom—which facilitates displacement of chlorine by (bio)nucleophiles—determines the observed biological properties. Reaction leads to an irreversible attachment of the molecule to a bionucleophile such as a DNA base or to a regulating protein. The modified bionucleophile is then the starting point for mutations or other malfunctions and also for chemotherapy against cancer. (Scheme 5)

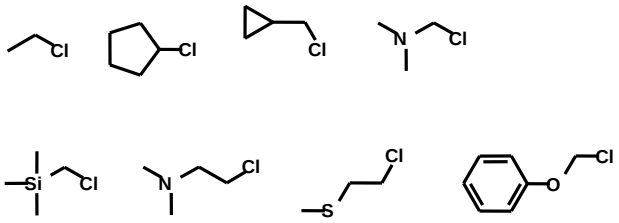


Scheme 2 - Biological activity of chemicals and time frame of action

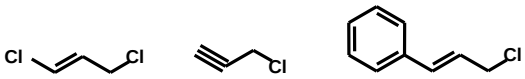
In principle the same holds true for any highly electrophilic sp^2 C-Cl bond like those found in acid chlorides, activated chloroheterocycles and certain aromatics, which have a halogen bond activated by electron withdrawing groups. Such chemicals, examples are listed in Scheme 3, are commonly used in the laboratory as intermediates for nucleophilic displacement reactions in chemical synthesis.

Scheme 3 - Some toxic reactive organochlorines useful as synthons in synthesis

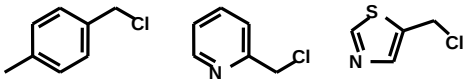
Alkylchlorides :



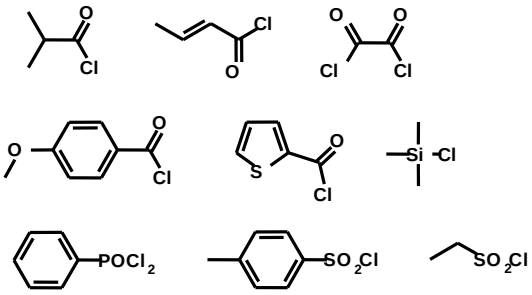
Allylchlorides :



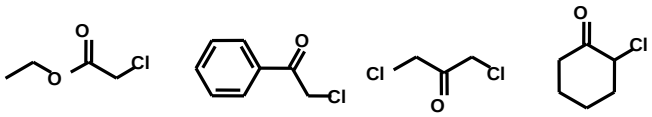
Benzylchlorides :



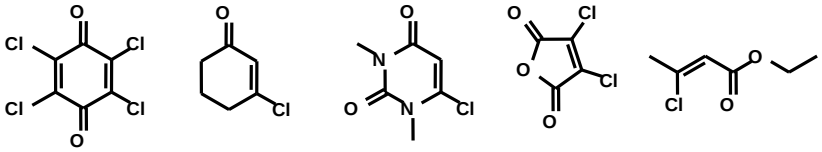
Acylchlorides :



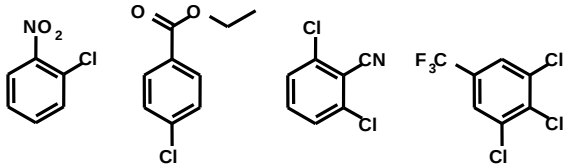
α-Carbonylchlorides :



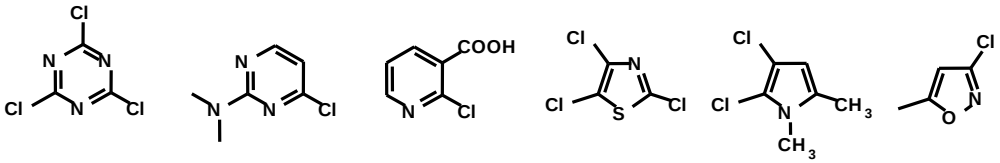
β-Ketonchlorides :



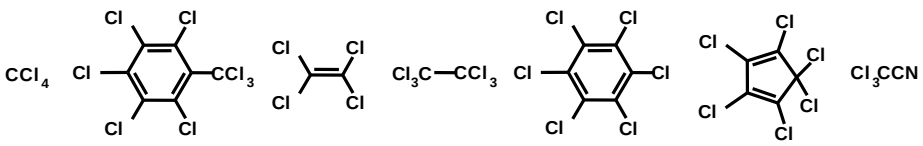
chlororeactive aromatic substances :

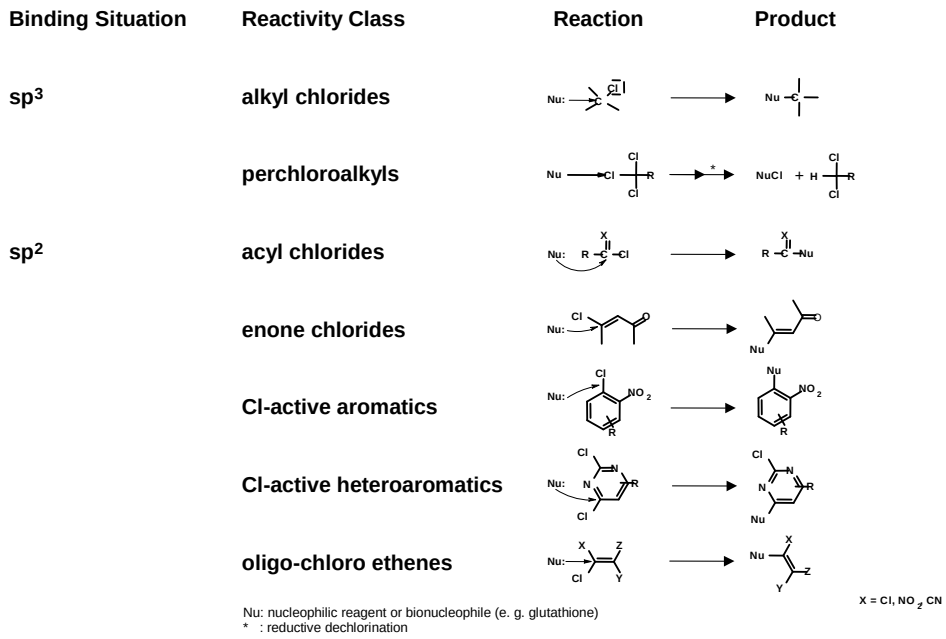


chlororeactive heteroaromatic substances :



perchloro-chemicals :

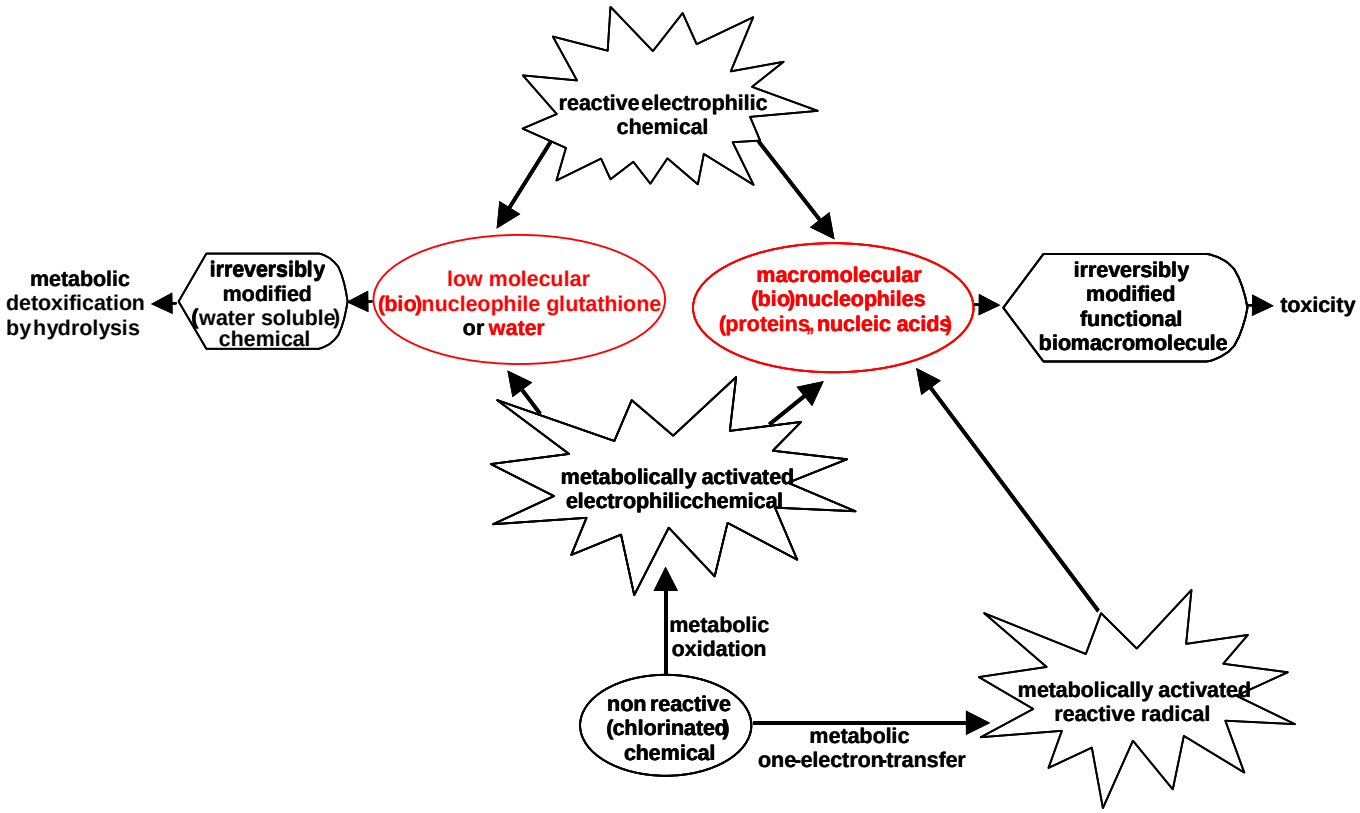




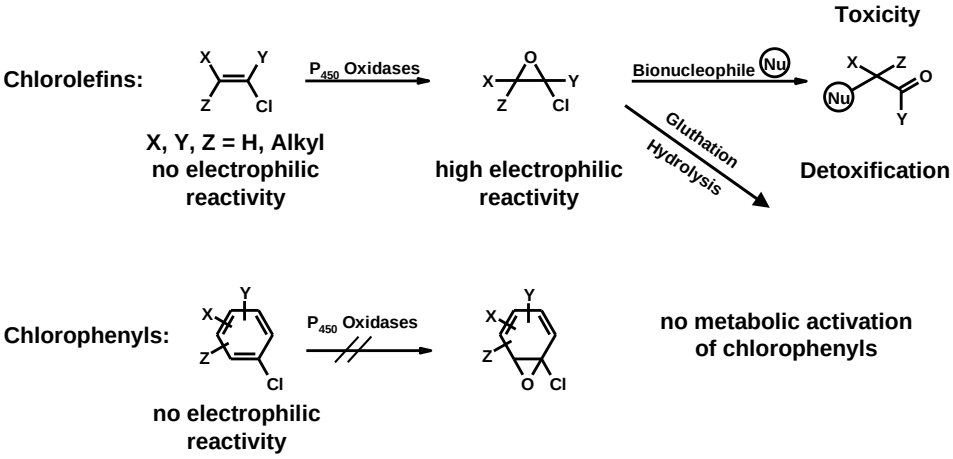
Scheme 4 - Examples of chemical reactivity of organochlorines

This electrophilic reactivity is also exploited metabolically by glutathione as a mechanism for detoxification and secretion or excretion in organisms that have taken up these chemicals (Scheme 4).

Another important metabolic step is the epoxidation, catalysed by oxygenases, of olefinic or aromatic double bonds to give more reactive electrophiles. These can then undergo irreversible reactions with bionucleophiles if they are not rapidly hydrolysed first. In the case of chlorinated olefins these epoxides are particularly reactive and may give rise to the effects reported by Henschler (Schemes 5 and 6).

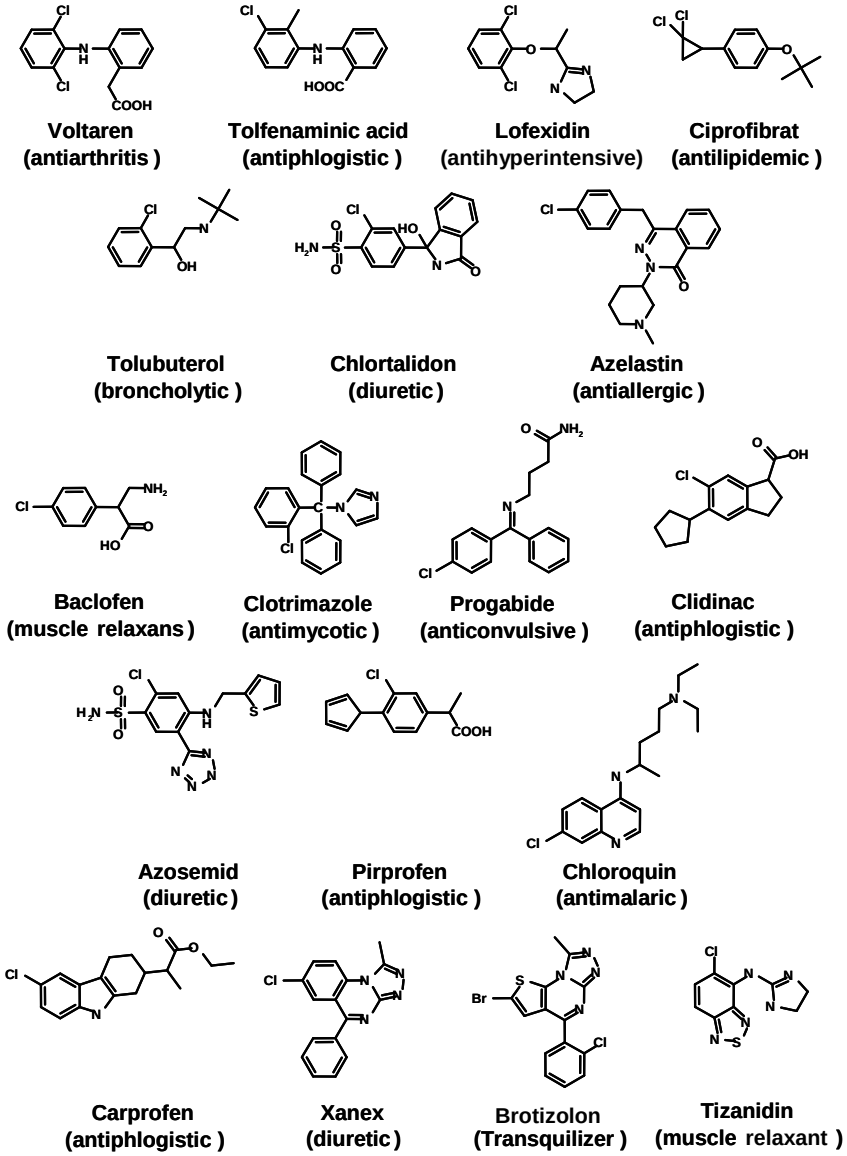


Scheme 5 - Reactivity of electrophilic chemicals in an metabolically active environment



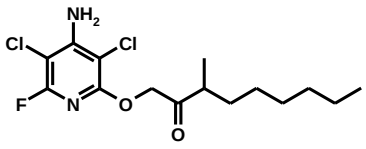
Scheme 6 - Metabolic activation: Important difference of chlorolefins and chlorophenyls according to Henschler

However, a chlorine atom at a nonactivated aromatic double bond diminishes reactivity, and here the formation of an epoxide and irreversible reactions with bionucleophiles do not take place [3] (Scheme 6).
This is an important observation because aromatic moieties play an important role in pharmaceutical drugs and pesticides, as shown in Scheme 7 and Scheme 8.

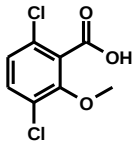


Scheme 7 - Some chlorinated pharmaceuticals

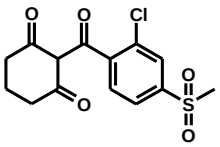
Herbicides



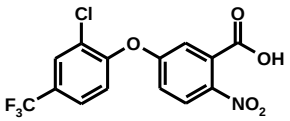
Fluroxypyrmeptyl



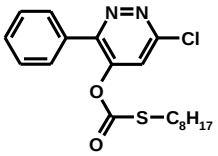
Dicamba



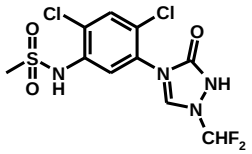
Sulcotrione



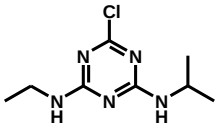
Acifluorfen



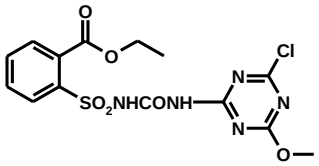
Pyridate



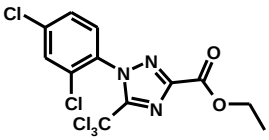
Sulfentrazone



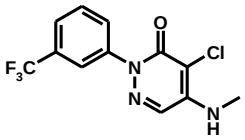
Atrazine



Chlorimuronethyl

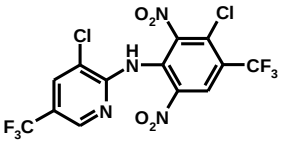


Fenchlorazole-ethyl

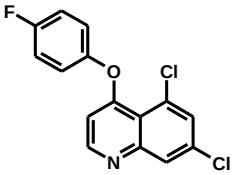


Norflurazone

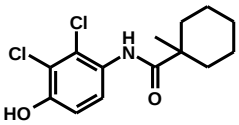
Fungicides



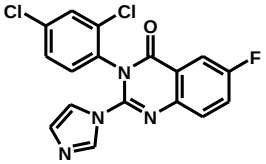
Fluazinam



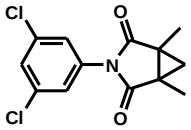
Quinoxyfen



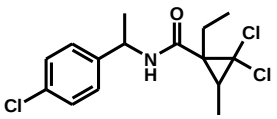
Fenhexamid



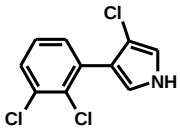
Fluquinconazole



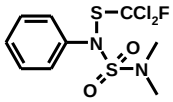
Procymidone



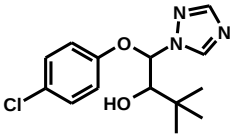
Carproamide



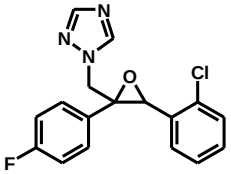
Fenpiclonil



Diclofluanid

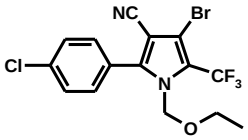


Triadimenol

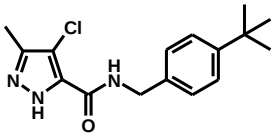


Epoxyconazol

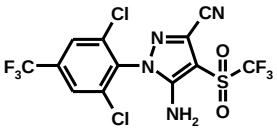
Insecticides/Acaricides



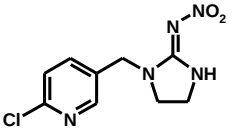
Chlorphenapyr



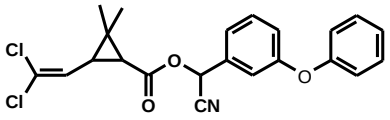
Tebuphenpyrad



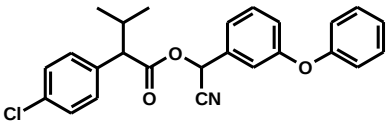
Fipronil



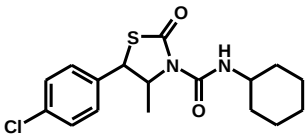
Imidacloprid



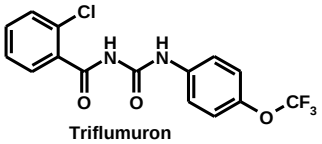
Cypermethrin



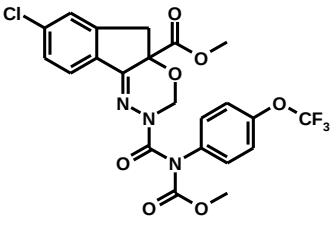
Fenvalerate



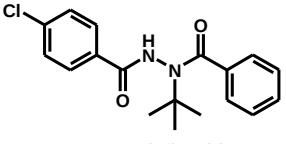
Hexythiazox



Triflumuron



Indoxacarb



Halofluozide

Scheme 8 - Some chlorinated commercial crop protection agents of the 1990s

In polychloroaliphatic systems like CCl_4, nucleophilic displacement is inhibited for electronic and steric reasons. In such cases, a single electron transfer reaction to the C-Cl bond by other radicals or metallo-enzymes leads to homolytic fission and the formation of reactive chlorine and carbon radicals. These radicals are detrimental to the function of biologically important proteins or nucleic acids (Scheme 5).

Such reactivity is well known to chemists and frequently exploited in synthesis where it is used to make new C-C bonds by radical addition of the polychloroaliphatic moiety across olefinic bonds.

In this review we shall not consider biological activity due to a high electrophilic reactivity of the C-Cl bond, which causes the irritant or toxic effects of the compounds reviewed by Henschler.

The mono-, oligo- and polychloro-alkanes and –alkanes, chloroquinones, allyl-type chlorides, benzyl chlorides, chloroheterocycles, α-chlorocarbonyl compounds and the like are the most important highly electrophilic intermediates used in chemical synthesis (Scheme 3).

3.2. Non-reactive chemical interactions with proteins and selective metabolism as the cause of specific biological activity of certain substances

The prime biological properties of drug or pesticide molecules with a molecular weight between 200 and 500 Daltons originate from the strong interaction with mainly protein target macromolecules. With the exception of insecticidal organophosphates and carbamates and carcinostatics against human cancer, this effect is caused by specific physical supramolecular interactions with a set of amino acid side-chains and peptide bonds rather than unselective chemical reaction in the classical sense. These phenomena may be hydrogen bonding, ion charge or dipole interactions, charge transfers, and hydrophobic or hydrophilic interactions. Such interactions occur at surfaces of proteins or in binding niches of protein pockets after water molecules have been displaced. The resulting changes in the protein function or conformation caused by binding to the effector molecule are the origin of the latter's biological activity. In most cases, the occurrence, structure and function of such functional proteins differ in detail markedly from plant to rat, from fungus to bacteria, etc, thus allowing the development of chemicals, such as herbicides, which interfere with only one kind of organism.

In the human body, bioactive compounds applied as a single dose or by short-term exposure are usually rapidly metabolised and thus rendered harmless and excreted as biologically unavailable derivatives (this can be demonstrated with radioactively labelled compounds). This metabolic power is different in plants, even in related plant species, microorganisms, insects and rats. This phenomenon, together with different protein binding niches, contributes to the occurrence of very selective compounds, like certain neurotoxic compounds, which interfere only with the neurosystem of insects but not with the nerves of warm-blooded animals. Imidachloprid **90** (Table 6) is one example.

However, a few substances with low chemical reactivity and high resistance to metabolisation, ecological persistence and lipophilicity, such as some polychlorinated substances (Scheme 1) have caused problems. As a result opponents of the chemical industry have targeted chlorine.

4. Chlorine Atoms as Substituents in Chemicals Intended to be Biologically Active

4.1. Physico-chemical properties of the chlorine substituent

The parameters of the chlorine substituent, relevant for biological and toxicological properties due to chemical reactivity of the C-Cl-bond as well as for non-binding interactions originate in the physico-chemical properties of the element chlorine proper and its position 17 in the periodic table of the elements.

1. The **electronegative nucleus** of the chlorine connected to a carbon atom withdraws electrons from other parts of the molecule, thus strongly polarising that bond causing a dipole moment. The tendency to become a chloride anion $Cl^{\ominus}$ with a complete 8 electron outer shell is the driving force for the strong reactivity of some C-Cl situations (Scheme 3; 4).
   In other cases one observes an increase of electrophilic reactivity at adjacent or more remote carbon centers not bound to the chlorine atom. Moreover, the loss of basicity of neighbouring nitrogen atoms, not only in heteroaromatics, as well as an increase of acidity of neighbouring or more remote O-H or N-H partial structures is observed.
   The σ-Hammett-constant of the Cl at the phenyl ring quantifies this influence for the case of benzoic acid. A polarisation of the aromatic σ-bond-framework by Cl causes a mesomeric +I-effect in the phenyl ring.

2. The electron cloud of the 3 nonbonding electron pairs around the chlorine nucleus enables the Cl-substituent to donate electrons into the π-system overlaying the σ-framework of a phenyl group, thus causing a mesomeric +M-effect, that is electron surplus at specific positions of the phenyl group. This is important for the orientation of electrophilic aromatic substitution reactions. On the other hand metabolic hydroxylation at the C-Cl-position is restricted (Scheme 6). The outer electrons are the origin of the London dispersion forces for non bonding supramolecular interactions (for example in proteins) with free electron pairs of other molecules or other electron densities like accumulations of C-H-bonds in aliphatic groups. This leads to strong lipophilic (=hydrophobic) properties of the Cl substituent locally and a strong increase of the overall lipophilicity of the whole molecule. This is a very important effect of chlorine in organochlorines of all kinds.

The geometric size of Cl measured as van der Waals radius is an important feature for steric effects of the Cl substituent particularly in the ortho position relative to another group of a phenyl ring. This leads to steric fixation of certain conformations necessary for supramolecular interactions with binding niches in biological macromolecules, like receptors. For many dynamic intermolecular interactions however the size of the electrostatic force field around a substituent calculated for a standard repulsion of an approaching standard charge may dominate over the smaller van der Waals radius.

The increase of lipophilicity of the whole molecule by a chlorine substituent leads to a higher partitioning of a chlorinated compound into the lipophilic phase of a cell membrane or lipophilic domains of a protein. This causes a higher local concentration of the compound near a biological target site, but, not necessarily a higher biological activity. The most important effect of a non-reactive chlorine atom in the biological activity of many compounds comes from chlorine as a substituent on an aromatic, heteroaromatic or olefinic moiety.

The properties mentioned above give rise to steric and/or electronic effects of the chlorine substituent(s) and lead to local electronic attraction or repulsion or to steric interference with any amino acid residue surrounding the position of the chlorine atom in the binding pocket of the protein. This in turn may cause a tighter interaction or a loosening of the contacts to the amino acids close to the chlorine or in other parts of the active molecule. Either one may affect the function of the target protein and cause an increase or decrease of biological activity. In other cases however a chlorine substituent may have no specific effect on the primary biological properties of the molecule to which it is attached.

A chlorine substituent is not a toxophoric group *per se*, except for the chemicals with a very reactive chlorine atom.

In addition to these properties, which are also connected to parameters mentioned below, the C-bonded chlorine has an oxidation potential. This enables certain microorganisms to cleave the C-Cl bond in a reductive manner under anaerobic conditions, as in naturally produced organochlorines in sediments [4].

4.2. Chlorine substituents and biological activity

It is an old observation that a chlorine substituent may cause a significant increase in substance's activity. This why fermentation broths are monitored for chlorinated compounds. However, many naturally occurring halogenated

compounds were discovered during structural elucidation of unknown compounds isolated from natural sources rather than identified because of their biological activity.

Chlorine can be introduced more or less easily to various positions of many chemicals that serve as precursors for synthetic biologically active compounds. The cases, in which a chlorine substituent at a given position of a molecule causes high activity, show up prominently in the literature on successful R & D projects on drugs and pesticides. Because of the high structural diversity, studies on inactive or less active congeners are lacking (understandable for economic reasons), it is not possible to deduce coherent rules to explain the change (improvement) in biological and toxicological properties caused by the chlorine substituents. This situation was deplored recently [2, 3]. There is not much information in the public domain on this issue.

The desire to have access to such material on more complex chlorinated compounds has also been expressed in a book review [6].

Nevertheless, for various reasons it has become common public opinion [7] that as a rule chlorine renders chemicals more toxic, and the more chlorine there is in the molecule, the greater its toxicity. **This generalisation is false.** The conclusion, deduced from about 120 small volatile, reactive, polychlorinated compounds, that non-carcinogenic properties of chlorinated compounds are the exception, has not been legitimately extended to chlorine-containing commercial pharmaceuticals and crop protection agents.

More than 300 compounds with low chlorine content have passed the stringent examinations required by the registration process, including thorough two-year chronic studies in experimental animals, and have been proved to be devoid of carcinogenic properties. In comparison, 50 % of the natural components of our daily food would have to be discarded as carcinogenic [8] if a positive *in-vitro* result in the Ames test (which checks mutagenicity towards some bacteria) had to be carried out—as is the case for testing commercial bioactive compounds. Some 50 % of isolated natural food components fed at maximum tolerated doses to test animals showed a carcinogenic potential. One should also consider here the relative degree of tolerance still shown by society in general to tobacco smoke (a proven carcinogen) and alcohol (a proven teratogen).

The intention of this article is to present examples from the literature as well as unpublished titbits from every-day industrial laboratory screening to show that the influence of chlorine on biological activity is rather complex.

The reality is that all chemists involved in drug and pesticide research who are searching for compounds with higher biological performance, investigate whether a chlorine atom (or another substituent) at the right position in the molecule renders it more active or more selective. In the first analysis the smaller the amount of a compound that is needed, the safer it is for humans and the environment and the fewer the resources consumed in its production. The more selective a compound is, the smaller the chance of unwanted side effects.

4.3. Chlorine substituents as an essential feature in some biologically active compounds

Amongst the known chlorinated, biologically-active ingredients used in medicine and agriculture, there is a group of compounds that owe their biological activity to the presence of all, or specific individual chlorine atoms attached to the molecule.

The old insecticidal polychloro compounds are one example. For less highly chlorinated or monochlorinated compounds there are also many examples where the chlorine-free analogue is practically devoid of biological activity (Table 3).

In the case of DDT **74** (Table 6) the presence of the CCl_3 moiety is essential to the activity of the pesticide. The two chlorine atoms must be in the 4,4'-positions. The corresponding 2,2'- and 3,3'-isomers are inactive as insecticides. This shows that chlorine has to be present in the molecule and in the right position. Nature discovered this principle long ago with its own chlorinated biologically active ingredients (Scheme 9). Some 1.5 % of all structurally known, natural compounds contain chlorine bound to a carbon atom. The chlorine substituent is essential for significant biological activity in a number of natural products such as the antibiotics clindamycin **1** [9], vancomycin **2** [10], chloramphenicol **3**, and griseofulvin **4** [11], and the antitumour compounds cryptophycin **5** [12], rebeccamycin **6** [13], clavulon **7** [14], neopyrolomycin **8** [15] and astin A **9** [16]. For natural azaphilons **10** [17] the chlorine exerts its effect on the endothelia receptor of rats and rabbits in quantitatively quite a different way. The chlorinated hydroxyketones **11** (Scheme 7) are important signaling compounds in slime moulds. The signal is switched off by enzymatic dechlorination [18].

The two chlorine substituents in vancomycin (**2**) induce a specific conformation that inhibits a bacterial enzyme [10]. This is probably an important role for chlorine atoms in many other biologically active compounds with complex structures.

Chloromethoxybenzyl alcohol **12** (Scheme 9), an important chlorinated trace compound ecologically, is produced by many fungi that degrade wood and other biomass. It serves as an important catalyst for intracellular H_2O_2 production. As substrate the non-chlorinated compound has a much lower binding to benzylalcohol oxidase, the producer of H_2O_2 [19], which causes the "cold combustion" of biomass such as fallen leaves.

Scheme 10 shows examples of chlorine-free compounds that are much less active at 0.1 % concentration (1,000 ppm). The increase in activity following introduction of chlorine substituents is associated with an increase in lipophilicity, leading to higher adsorption to proteins such as albumins [20], glucosidase [21] or other enzymes. The very strong increase in sweetness of sucralose **13** [22], the trichloro analogue of saccharose, can be explained in this way. In addition, due to the electronegativity of chlorine, neighbouring NH or OH bonds maybe acidified, giving rise to an anionic species, which, when combined with high lipophilicity, is capable of shuttling protons across the cell membrane. This causes the proton gradient across the membrane to collapse and an end to energy production in the cell by uncoupling of oxidative phosphorylation [23].

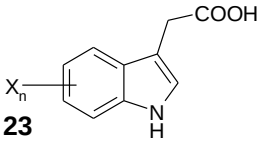
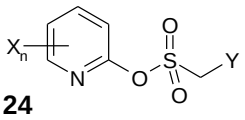
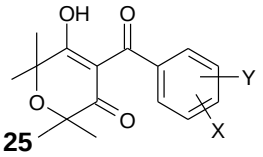
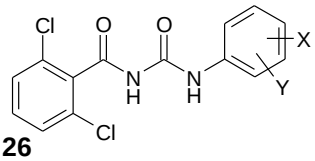
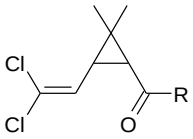
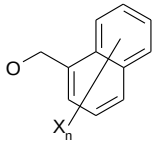
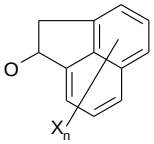
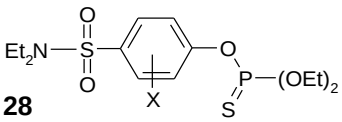
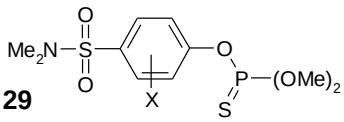
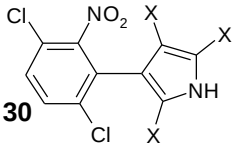
For chlorophenols **14**, high adsorption to albumin is correlated with high adsorption to mitochondrial proteins and inhibition of phosphorylation; the inhibition factor increases by a factor of 100 as chlorine content [24] increases from Cl_1 to Cl_5.

The weak fungicidal activity of benzylalcohol **15** is improved by a factor of a hundred [25] by increasing the number of Cl atoms from zero or one chlorine atom to three chlorine atoms.

The inhibition of the influenza virus by benzotriazoles **16** is steadily enhanced by a factor of 1,200 through introduction of Cl in the 5, 6-, then 4, 6-, followed by the 6-position up to the tetrachloro derivative [26]. However, in the chloroindole series **17** the herbicidal efficacy has a clear optimum with a Cl in the 7-position. The 3-monochloro, and the 2, 3- and 5, 7-dichloro derivatives are much less active; the pentachloro derivative is inactive [27]. In the case of chlorinated benzoquinones **18**, the increase in activity going from parent quinone to tetrachloroquinone is much less pronounced [25].

Salicylic acid is an important signalling compound in plants and a regulator of oxidative events in mammals. Its *O*-acetyl derivative is pharmacologically important in humans. It is also an inducer of genes in plants, when taken up by the roots from a 10^{-5} M solution.

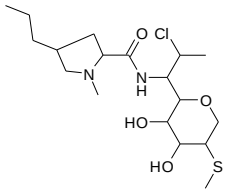
Table 3 - Chlorine substituents as modulators of biological activity (X = H, Cl)

Compounds	Biol. Effect	Structure-Activity-Relation Substituents X,Y	Ref.
 **23**	X = H, Cl Natural plant growths hormones 4-Cl: „death hormone?“	4Cl > 5,6Cl_2 >> H > 4,7Cl_2 > 5,7Cl_2 > 7Cl	[27]
 **24**	Nematicide	3,5Cl_2; Y=H > 5Cl; Y=Cl > 6Cl Y=Cl > 3,5Cl_2; Y=Cl	[37]
 **25**	Herbicide	2NO_2; 4Cl > 2,4Cl_2 > 2NO_2, 3Cl > 4NO_2; 2 Cl	[38]
 **26**	Insecticide	2CH_3; 4Cl > H >> 3CH_3; 4Cl - inactive 2CH_3; 4,5Cl_2 - inactive	[39]
 **27**	Insecticide		[40]
R= 		Cl_2 > H	
		H > Cl_2	
 **28**	Insecticide: Mouse toxicity:	3Cl > 2Cl > H H = 2Cl = 3Cl; 30 mg/kg	[41]
 **29**	Insecticide: Mouse toxicity:	2Cl = 3 Cl > H H (9.5 mg/kg) = 2Cl > 3Cl (48 mg/kg)	[41]
 **30**	Natural bactericide	4,5Cl_2 > 2Cl > 2,5Cl_2 > 5 Cl = 2,4,5Cl_3 > 2,4Cl_2	[42]

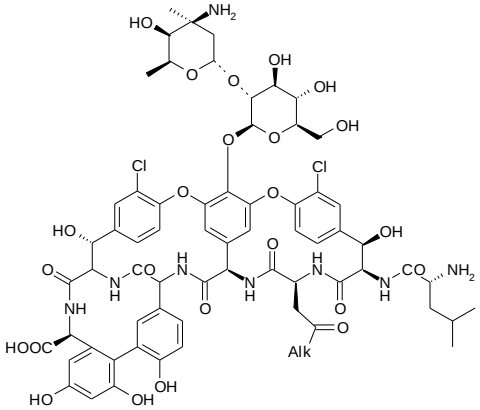
Introduction of a 5-chloro substituent **19** renders salicylic acid ten times more active [28]. However, the corresponding *O*-methyl and *O*-acetyl derivatives are both inactive. This indicates a physiologically important increase in acidity of the free phenolic or carboxylic group, combined with the expected increase in lipophilicity.

Inhibition of the Hill reaction, crucial in plant photosynthesis, by *N*-phenyl-*O*-propyl-carbamates **20** increases 100-fold from the unchlorinated to the 3, 4-dichloro derivative [29]. But in the case of the inverse *N*-methyl-*O*-phenyl carbamates **21**, which are insecticidally active, a chlorine atom in the 4-position has no additional effect on inhibition of acetylcholine esterase.

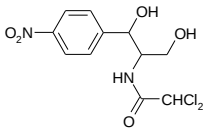
However, a chlorine substituent in the meta-position causes a fourfold increase in inhibition [30], demonstrating that a third effect is operating in addition to increases in acidity and lipophilicity, namely the structural factor of chlorine substitution. The same effect is observed in the case of bactericidal phenylsulfanilides **22** [30].



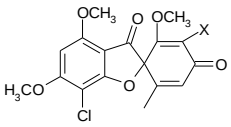
1 Clindamycin



2 Vancomycin

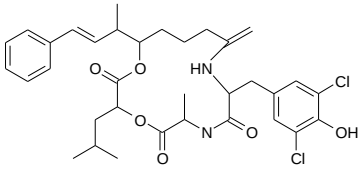


3 Chloramphenicol

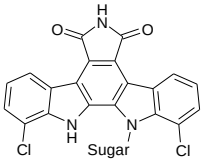


X = Cl / H

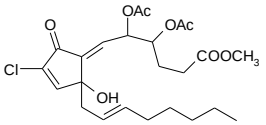
4 Griseofulvin



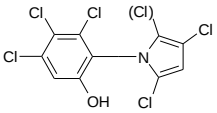
5 Cryptophycin



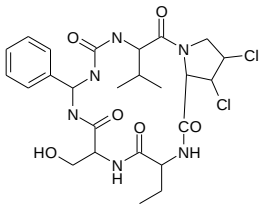
6 Rebeccamycin



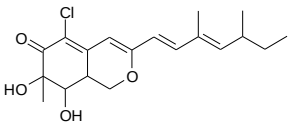
7 Clavulon



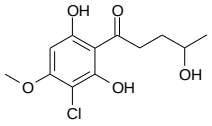
8 Neopyrolomycin



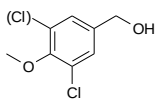
9 Astin A



10 Azaphilon

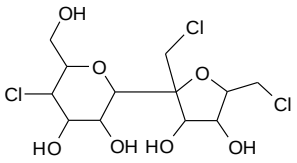


11

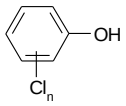


12

Scheme 9 - Naturally occurring chlorinated biologically active compounds, where chlorine is necessary for activity

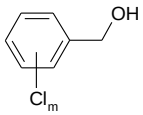


13 Sucralose



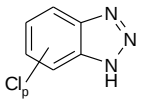
n = 0 - 5

14



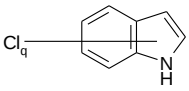
m = 0 - 5

15



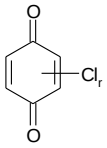
p = 0 - 4

16



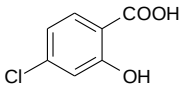
q = 0 - 5

17

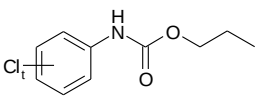


r = 0 - 4

18

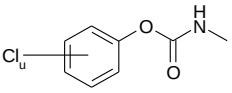


19



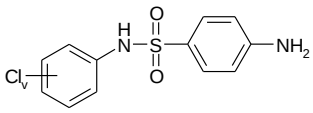
t = 0 - 2

20



u = 0 - 2

21



v = 0 - 2

22

Scheme 10 - Chlorine substituents as enhancers of biological activity

Another structural factor, steric interference at the ortho-position of chlorophenols renders the ortho-chlorophenol less toxic to water fleas than derivatives having the same number of chlorine atoms but with free ortho-positions [31].

4.4. Chlorine as one of the many substituents modulating biological properties

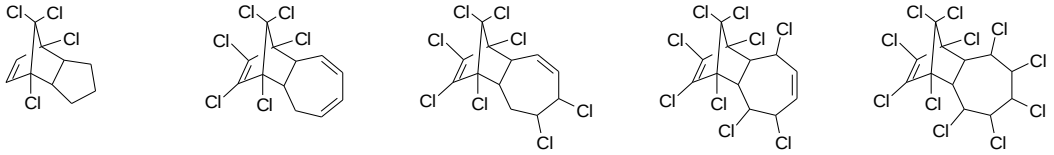
As shown above, chlorine may be essential for a compound to have any biological activity. In other examples it enhances the weak intrinsic biological activity of the parent molecule (Scheme 10). Most of the commercial chlorine-containing pharmaceutical and crop protection agents, either isolated from natural sources or invented in synthesis laboratories, have been selected for this reason (Schemes 7, 8). These compounds are neither chemically reactive nor uncouplers. Their biological effect originates in a non-bonding “lock and key” interaction with a specific binding niche in an enzyme, receptor or other functional protein, either cytosolic or membrane bound. Thus they act by a specific biochemical mechanism that is not just caused by a general biophysical effect or by chemical reactivity.

In such cases the position of the chlorines on the molecule determines the magnitude and kind of biological effect *in-vitro* or *in-vivo*. It must be mentioned here that chlorine is just one out of a variety of substituents that are tried out in the course of lead structure optimisation or a lead structure search. This arsenal stretches from a methyl pattern to a *t*-butyl group, from phenoxy to heterocyclic moiety, from fluorine to a sulfamoyl group and so on, and encompasses hydrophilic amino and hydroxy groups, piperazinyl, morpholinyl and other basic moieties.

The compounds shown in Table 3 have an optimal number and/or substitution pattern of chlorine atoms to give the best activity. It is not just the presence of a chlorine atom that causes biological activity. The whole molecule is involved, whereby some structural features are more or less variable and others are essential.

The relative contributions of each substituent to the measured bioactivity can be derived by a quantitative structure-activity relationship (QSAR) calculated from a series of close analogues.

A typical example demonstrates how the replacement of a hydrogen atom by chlorine affects the structure and changes biological activity.



139 140 141 142 143

Scheme 11 - Diminishing insecticidal activity with increasing chlorine content in Chlordane analogues.

Analogues of the natural bactericide pyrrolomycin A **30** (Table 3) were investigated. The ranking of the bactericidal activity of the differently chlorinated homologues showed no correlation with the degree of chlorination.

Table 4 - Shift in fungicidal activity by chlorination

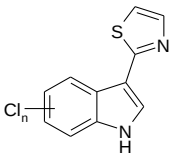
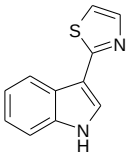
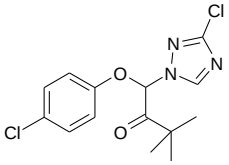
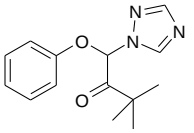
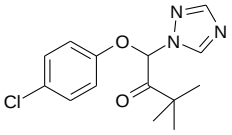
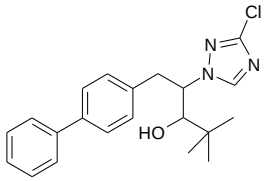
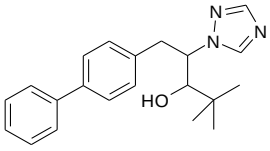
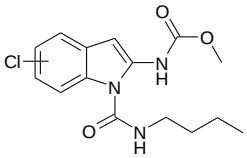
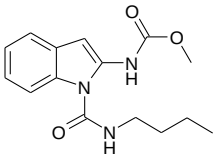
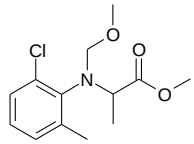
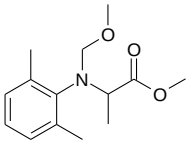
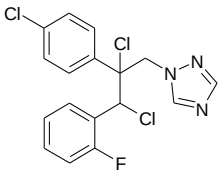
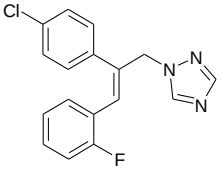
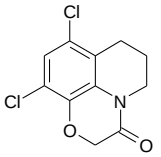
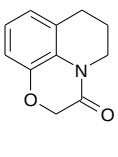
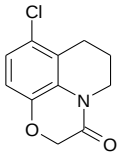
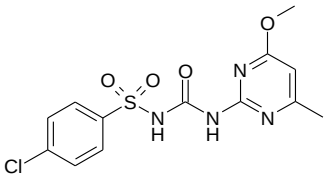
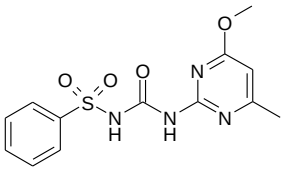
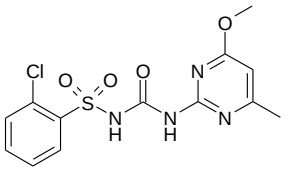
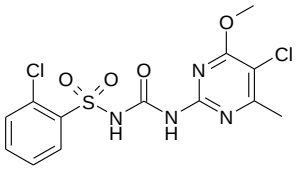
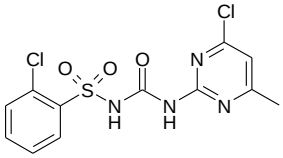
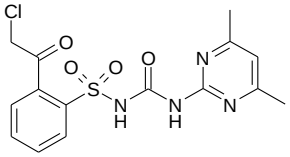
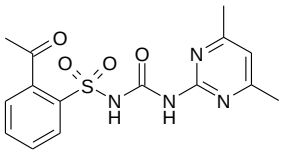
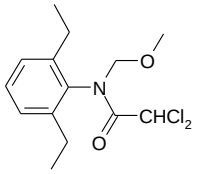
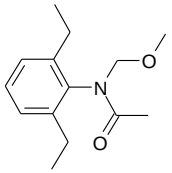
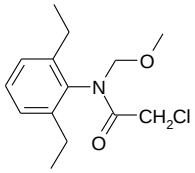
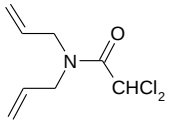
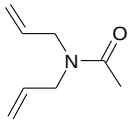
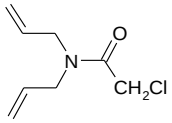
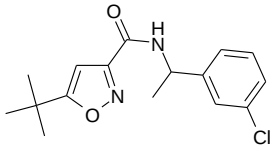
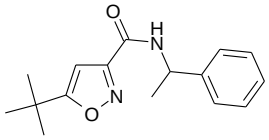
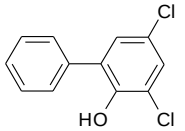
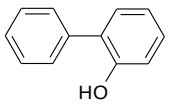
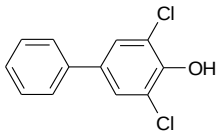
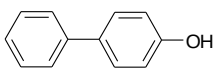
Less Active or Inactive Chlorinated Derivatives	Activity	Parent Compound	Activity	More Active Chlorinated Derivative	Activity	Ref.
 **31**	-	 **32** Natural Camalexin	+			[43, 44]
 **33**	-	 **34**	++	 **35** Triadimefon	+++	[44]
 **36**	-	 **37**	++++			[44]
 **38**	-	 **39** Benomyl	+++			[44]
 **40**	-	 **41** Metalaxyl	+++			[44]
 **42**	+	 **43**	++			[44]
 **44**	+	 **45**	++	 **46**	++(+)	[40]

Table 5 - Shift in herbicidal activity by different chlorine substitution pattern in some herbicide classes

Less Active or Inactive Chlorinated Derivatives	Activity	Parent Compound	Activity	More Active Chlorinated Derivates	Activity	Ref.
 **47**	-	 **48**	+	 **49** Chlorsulfuron	++++	[44]
 **50**	-					
 **51**	(+)					
 **52**	-	 **53**	+++			[44]
 **54**	-	 **55**	-	 **56** Alachlor	+++	[44]
 **57**	- Protects against herbicide damage	 **58**	-	 **59** Allidochlor	++	[44]
 **60**	-	 **61**	++			[44]
 **62**	+	 **63**	++ Rice root inhibition			[45]
 **64**	(+)	 **65**	++ Rice root inhibition			[45]

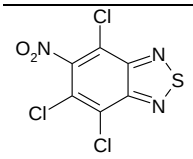
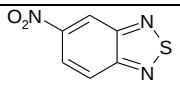
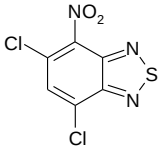
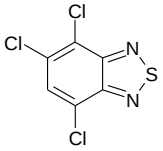
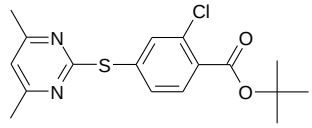
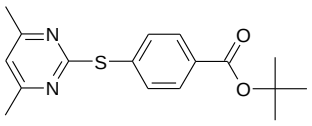
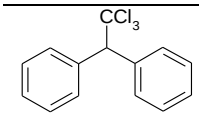
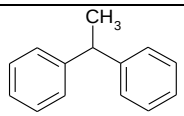
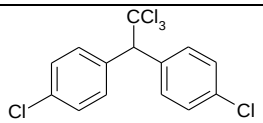
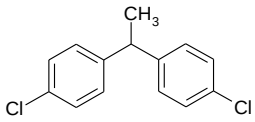
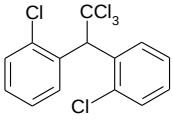
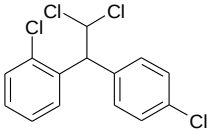
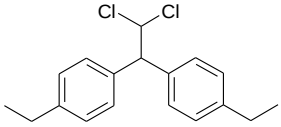
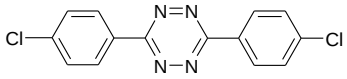
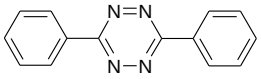
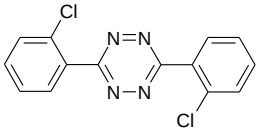
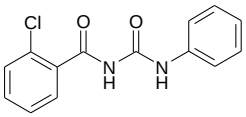
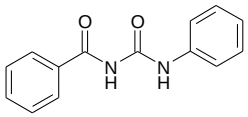
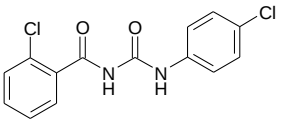
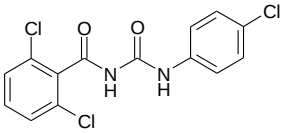
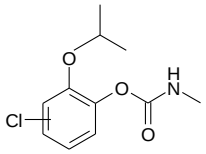
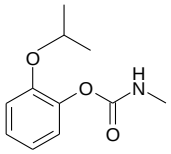
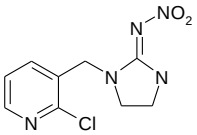
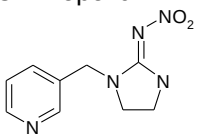
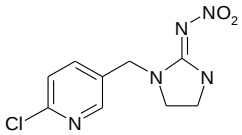
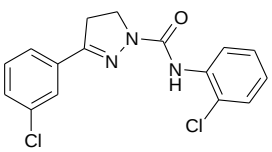
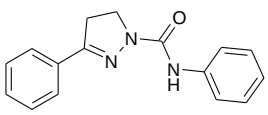
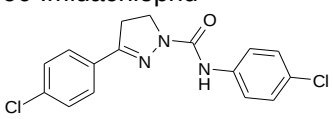
Less Active or Inactive Chlorinated Derivatives	Activity	Parent Compound	Activity	More Active Chlorinated Derivates	Activity	Ref.
 **66**	+	 **67**	++			[46]
 **68**	-					
 **69**	-					
 **70**	(+)	 **71**	++			[44]

Table 6 - Shift in insecticidal activity by different chlorine substituent pattern in various classes of insecticides

Less Active or Inactive Chlorinated Derivatives	Activity	Parent Compound	Activity	More Active Chlorinated Derivatives	Activity	Ref.
 **72**	-	 **73**	-	 **74** DDT	++++	[44]
 **75**	-					
 **76**	-					
 **77** Mitotane	Anti-tumor activity	 **78** Pertane	++			[54]
 **79**	-	 **80**	-	 **81** Clofentezine	+++	[44]
 **82**	-	 **83**	-	 **84**	++	[44]
 **85**	-					
 **86**	-	 **87** Propoxur	+++			[44]
 **88**	(+)	 **89**	+	 **90** Imidachloprid	+++ +	[44]
 **91**	-	 **92**	-	 **93**	++	[44]

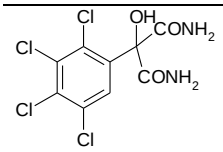
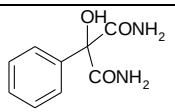
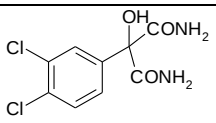
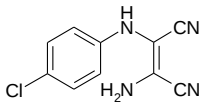
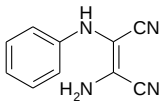
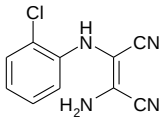
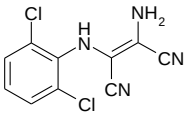
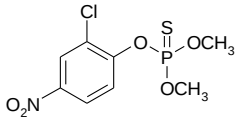
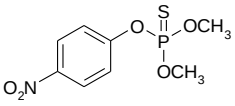
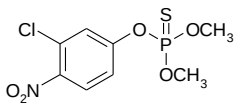
Less Active or Inactive Chlorinated Derivatives	Activity	Parent Compound	Activity	More Active Chlorinated Derivatives	Activity	Ref.
 **94**	-	 **95**	(+)	 **96**	++	[44, 55]
 **97**	-	 **98**	-	 **99**	+++	[44, 56]
 **100**	-					
 **101**	+ LD_{50} 40 mg/kg	 **102** Parathion	+++ LD_{50} 25 mg/kg rat.			[44]
 **103**	++ LD_{50} 880 mg/kg					

Table 7 - Influence of chlorine substituent on insecticidal activity of pyrethroids [47]

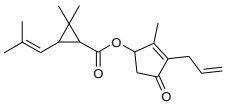
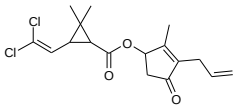
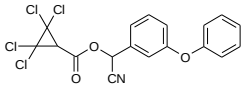
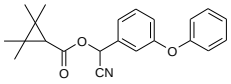
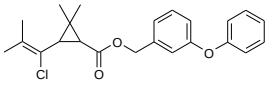
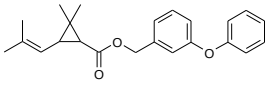
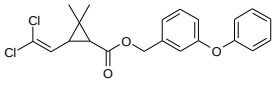
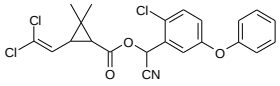
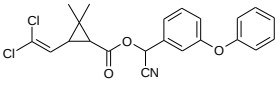
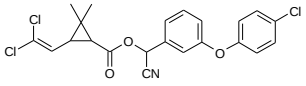
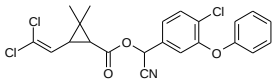
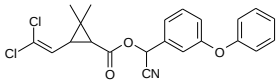
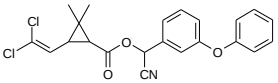
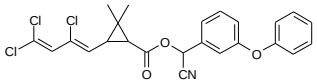
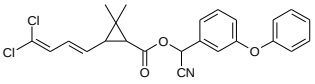
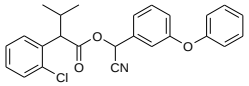
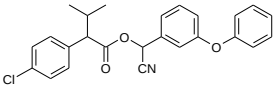
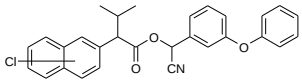
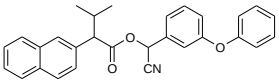
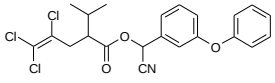
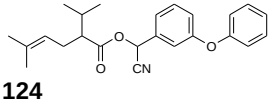
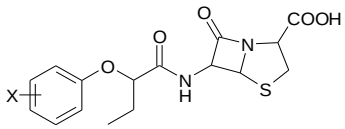
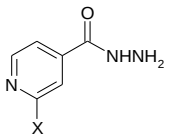
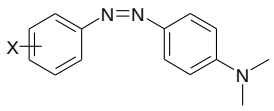
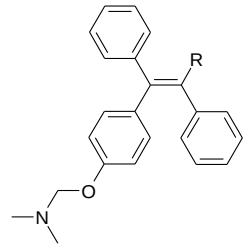
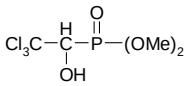
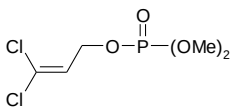
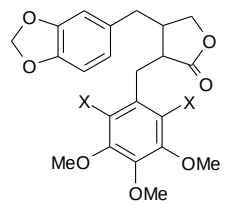
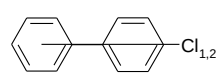
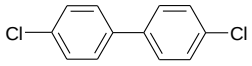
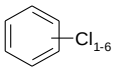
Less Active or Inactive Chlorinated Derivatives	Activity	Parent Compound	Activity	More Active Chlorinated Derivatives	Activity
		 **104**	++	 **105**	+++ knock down
 **106**	-	 **107**	+++		
 **108**	-	 **109**	+++	 **110**	++++
 **111**	-	 **112** (I) cis/trans	++++	 **113**	++++ not changed
 **114**	-				
 **115** S trans αR enantiomer	-	 **116** R trans αS enantiomer	+++++		
 **117**	+	 **118**	+++		
 **119**	(+)	 **120**	+++		
 **121**	-	 **121**	++		
 **123**	-	 **124**	+		

Table 8 - Diminution of biological activity of drugs and other chemicals by chlorination

Active Compound (X = H)	Property	Activity	Less Active Chlorinated Derivative (X = Cl)	Activity	Ref.
 **125**	Bactericide	$\lg \frac{1}{C}$ 5.9	2Cl $2,5Cl_2$ $2,3,6Cl_3$ Cl_5	$\lg \frac{1}{C}$ 5.4 5.2 4.7 4.2	[48]
 **126**	Tuberculostatic	I_{max} 10^{-6}	2Cl	I_{max} $4 \cdot 10^{-6}$	[49]
 **127**	Carcinogen	Rel. activity 6	3Cl 2Cl 4Cl $2,5Cl_2$ $2,4,6Cl_3$	Rel. act.: 5-6 2 1-2 inact.	[50]
 $R = C_2H_5$ **128**	Carcinogen (rat liver)		R = CH_2CH_2Cl **129**	Not carcinogenic	[57]
				Not carcinogenic	[44]
			 1**30**	Not carcinogenic	[44]
 **131**	Antitumoral		$2,6Cl_2$	Less active	[51]
 **132**	Kidney damage in rats		Higher chlorinated congeners	Less damage	[52]

Active Compound (X = H)	Property	Activity	Less Active Chlorinated Derivative (X = Cl)	Activity	Ref.
 **133**	Persistent in chicken		Higher chlorinated congeners with free 4,4‘ position	Less persistent	[53]
 **134**	Leucemic		 **135**	Not leucemic, not carcinogenic, not genotoxic	[58]

4.5. Chlorine substituents diminish or abolish biological activity

Researchers often investigate whether chlorine will optimise activity of a compound. However, a chlorine atom in all or in specific positions of a biologically active lead compound can diminish or even abolish biological activity. Such compounds are of no further commercial interest.

As chemists are only looking for an improvement in biological activity and/or toxicological or environmental properties, it is not easy to retrieve information about these diminished effects. Failures are usually consigned to oblivion. Information on chlorine as a diminisher of biological activity is not interesting for these experts and not worthy of publication. The author has managed to compile a number of examples (Tables 4 – 8). These are drawn from his own results gathered during insecticidal research, from a rather old private archive [32], and through enquiries amongst colleagues in the agrochemical division of his institution. These clearly demonstrate the modifying effect of a chlorine substituent on a parent compound (or lead structure, if active) in both directions—to biologically more active or to less active compounds.

An old result in a screen against six different insect species shows that insecticidal activity in the chlordane analogue series does not increase with further chlorination (Scheme 11) [33]. The author has examples from his involvement in pyrethroid research showing how the number of chlorine substitutions at specific positions of an active molecule can increase, decrease or have no impact on insecticidal activity (Table 7).

5. Sources

In principle information on the influence of chlorine atoms on biological activity can be retrieved from industrial chemists working on lead structure optimisation in about 100 life science companies. In the past this has not been a topic of particular interest. However, it is hoped that more collectors of such screening data will exchange this information at future conferences or submit it for publication.

This review is intended to initiate a scientific discussion about sharing information with the public, especially as the subject is not very exciting scientifically-speaking. However, it is an important contribution to the general knowledge of practical chemistry regarding the biological properties of chemicals. This knowledge would be of great value for sound judgement at the interface of science, commerce and politics and redress the balance against recent scientifically nonsensical statements that have been made about chlorine.

6. Summary and Outlook

The influence of chlorine on biological activity of chemicals can be grouped in the following way:

Group 1: Chemically very reactive electrophilic mono and oligo organochlorines of lower molecular weight (< 200). These are synthons for synthesis such as alkylating agents, acid chlorides, chlororeactive aromatics and heteroaromatics. In addition small molecules with a high chlorine content and low electrophilic reactivity are used as solvents and are also useful reagents in radical reactions. The German MAK list includes 150 of such chemicals that are mutagenic, carcinogenic and/or allergenic potential and require control in the workplace. These examples are scientifically well-documented (Henschler´s book "Toxicity of Organochlorines" [3]). None of these reactive chemicals or solvents is used intentionally for biological properties.

Group 2: Chemically very inert, persistent, polychlorinated and highly lipophilic, bioaccumulating chemicals (molecular weight 200 – 500), having carcinogenic and/or estrogenic properties, belonging to the so-called "dirty dozen" list of Persistent Organic Pollutants (POPs). These include polychlorodibenzdioxins, polychlorobiphenyls, polychlorophenols, fluorochlorocarbons and perchloroaliphatics. Some had important technical uses; others are unwanted technical side- or trace products. A few were important insecticides in the past. The use of these chem.icals is now banned or severely restricted in Western Europe and the US.

Group 3: Monochloro- or oligochloro-organics with a high number of carbon atoms (molecular weight 250 – 1,200). The chlorine substituent in these naturally-occurring or synthetic chemicals is crucial for the observed biological activity. Examples include natural antibiotics and pesticides.

Group 4: Group 4: Monochloro- or oligochloro-organics with a high number of carbon atoms (molecular weight 200 – 600). Chlorine substituents at specific positions in these chemicals increase or decrease an intrinsic activity several fold.

Examples include useful drugs or pesticides, which have low acute toxicity and have been thoroughly investigated in two-year chronic studies on live mammals (and not just *in-vitro* on enzymes or bacteria) at maximum tolerated doses for carcinogenic, long-term effects and other detrimental and prohibitive properties. Applying the highest available scientific standards for evaluation of pesticides and drugs, the German authorities had approved about 140 chlorinated pesticides [34] and about 120 chlorinated pharmaceuticals in Germany as of 1992 [35]. About 330 chlorinated pharmaceutical drugs are listed in the 1996 Merck Index [36].

These figures do not include reactive cytostatics, disinfectants and chloride salts. This is proof that such compounds are neither carcinogenic nor toxic at levels relevant to the extended scope of practical use.

Group 5: Monochloro- or oligochloro-organics with a high number of carbon atoms and low chlorine content (molecular weight 200 – 600). Chlorine substituent(s) at specific positions of the parent molecule diminish or abolish some or all facets of a lower or higher biological activity. It can happen that no position is found where the chlorine substituent preserves or increases activity. Examples include unsuccessful and usually unpublished results from industrial drug and pesticide research. There are about 70 cases presented in this review from one institution—this certainly represents a small part of what is known. An in-depth literature survey would yield many more examples.

Group 6: Chlorine-containing chemicals (molecular weight 200 – 800) with a high diversity of structures and wide range of physico-chemical properties that show no biological activity in primary screens involving many in-vitro and in-vivo tests, including toxicologically relevant target proteins. Examples include many tens of thousands of test compounds submitted for high-throughput screening in the life-science industry.

Conclusion

Neither biological experiments nor experience provide justification for deducing a general rule that chlorine renders a compound more toxic or more active *per se*. Whether this is true or not has to be found out empirically for each compound in case-by-case studies. Many surprises line the path of the history of the development of biologically active compounds. Compounds that turn out to be unsafe for use as a drug or pesticide are discarded, whether they contain chlorine or not.

Each chlorinated compound tested in the process of lead structure optimisation for better drugs or pesticides, has the chance of being found to be more active, more selective, safer and more benign for humans and the environment. It would be a serious professional neglect for a synthesis chemist to refrain from using a chlorine substituent in such optimisation work on a lead structure.

In the past, and even today, lead structure work on many novel types of chemicals of biological interest may have been prematurely classified as unfruitful because the structures were not probed at the proper positions with the right substituents, of which chlorine is but one.

Acknowledgements

I thank K. H. Büchel for providing his collection of structure/activity data from the 1960s/1970s. The help of my fellow chemists in the Central Research Laboratory and Crop Protection Research at Bayer AG in providing data is appreciated. I thank Michaela Willms for preparing the manuscript and Graham Holmwood for his constructive critical reading for content and grammar of this paper.

References

[1] B. Hileman, C + E News, April 19, **1993**, 11
[2] O. Henschler, Angew. Chem. **1994**, *106,* 1997
[3] O. Henschler, Toxikologie Chlororganischer Verbindungen, Verlag Chemie Weinheim, **1994**, 195
[4] G. Müller, G. Nkusi, F. Schöler, J. prakt. Chem. **1996**, *338,* 23
[5] S. L. Neidleman, Crit. Rev. in Microbiol., Cleveland, **1975**, *5,* 333
[6] H. Parlar, Naturwissenschaften **1995**, 82, 390
[7] H. Tügel, Geo **1996**, (10), 158
[8] B. Ames, R. Magaw, L. S. Gold, Science **1987**, 232, 271; ibid. **1990**, 249, 970
[9] R. D. Birkenmeyer, F. Kagan, J. Med. Chem. **1970**, *13*, 616
[10] C. Harris, R. Kannan, H. Kopecka, T. M. Harris, J. Am. Chem. Soc. **1985**, *107,* 6652
[11] R. Crosse, R. McWilliams, A. Rhodes, J. Gen. Microbiol. **1964**, *34,* 51
[12] T. Golakoti, J. Ogino, C. E. Heltzel, L. Le Husabo, C. M. Graig, L. K. Larsen, G. Patterson, R. E. Moore, S. L. Mooberry, J. Am. Chem. Soc. **1995**, *117,* 12030
[13] J. A. Bush, B. H. Long, J. J. Catino, W. T. Bradner, J. Antibiot. **1987**, *40,* 668; ref. [5], p. 161
[14] Y. Yamada, I. Nagoka, Tetrahedron Lett. **1985**, *26,* 5053
[15] K. Tatsuda, M. Itoh, J. Antibiot. **1994**, *47,* 262
[16] H. Morita, J. Nagashima, Y, Uchiumi, O. Kurobi, K. Takeda, H. Itokawa, Chem. Pharm. Bull. **1996**, *44,* 1026
[17] Y. Tanaka, K. Matsuzaki, C. L. Zhong, H. Yoshida, T. Kawakubo, R. Masuma, H. Tanaka, S. Omura, I. Antibiot. **1996**, *49,* 1956; R. Pairet, S. K. Wrigley, J. Chettland, E. E. Reynolds, M. A. Hayes, J. Holloway, A. M. Anisworth, W. Katzer, X. H. Cheng, J. Antibiot. **1995**, *49,* 913
[18] P. Morandini, J. Offer, W. Graham,, R. R. Kay, Biochem. J. **1995**, *306,* 735
[19] E. de Jong, J. A. Field, A. E. Cazemier, J. A. M. de Bont in ref. [4], p. 251
[20] W. Scholtan, Arzneimittelforschung **1963**, *13,* 347
[21] C. Hans, E. W. Deutsch, R. N. Schmith, J. Am. Chem. Soc. **1960**, *87,* 2738
[22] M. Mathlouthi, A.-M.Seuvre. G. Birch, Carbohydrate Research **1986**, *152,* 47
[23] K. H. Büchel, W. Draber, Progress in Photosynthesis Res. **1969**, *3,* 1777
[24] C. Hansch, K. Kiehs, G. L. Lawrence, J. Am. Chem. Soc. **1965**, *87,* 557; M. Stockdale, M. J. Selwyn, Eur. J. Biochem. **1971**, *21,* 565
[25] C. Hansch, E. J. Lien, J. Med. Chem. **1971**, *14,* 653
]26] J. Tamm, R. Bablanian, M. H. Nemes, C. H. Shunk, F. M. Robinson, K. Folkers, J. Exp. Medicin **1961**, *113,* 625
[27] K. C. Engvild in Naturally Produced Organohalogenes (Eds. A . Grimvall, E. W. B. de Leer), Kluwer Academic Publishers Dortrecht **1995**, p.227
[28] C. Lechelt-Kunz, Central Re. Bayer AG, (**1992** unpubl.)
[29] N. D. Camper, O. E. Moreland, Biochem. Biophys. Acta **1965**, *94,* 383
[30] C. Hansch, Intra. Sci. Chem. Rept. **1974**, *8* (4), 17
[31] J. Devillers, P. Chambon, Bull. Environ. Contam. Toxicol. **1986**, *37,* 599
[32] K. H. Büchel, Agrochem. Div. Bayer AG, Collection of Structure Activity Data 1965 – 1975, private communication
[33] K. H. Büchel, A. Conte, Birlinghofen, Screening results (**1966** unpubl.); Chem. Ber. **1967**, *100,* 863; **1966**, *101,* 99
[34] Farm Chemical Handbook, Meister Publ. 1996
[35] Rote Liste, **1992**, Germany
[36] Merck Liste, **1996**, 13th Edition
[37] M. Harmer, C. W. Huffman, S. S. Ristich, J. Agric. Food Chem. **1970**, *18,* 57
[38] U. K. Pat. 2 205.316, Sandoz Co. Chem. Abstr. XXX, Poster at Congress on Heterocyclic Chemistry, Taipei, Aug. 1995
[39] K. Wellinga, R. Mulder, J. J. van Daalen, J. Agric. Food Chem. **1973**, *21,* 348
[40] K. Naumann, Agrochem. Div. Bayer AG, Screening results (1977 unpubl.)
[41] S. Suzuki, J. Miyamoto, K. Fujimoto, H. Skamoto, Y. Nishizawa, Agr. Biol. Chem. **1970**, *34,* 1697
[42] S. Neidlman, Biohalogenations, Ellis Horwood, Chichester 1986, p. 121
[43] H. Wamhoff, A. Franken, Univ. Bonn (1993 unpubl.); L. M. Browne, K. L. Conn, W. A. Ayer, J. Tewary, Tetrahedron **1991**, *47,* 3909
[44] Bayer AG, Screening results (unpubl.)
[45] H. M. Cheng, M. Eto, S. Kuwatzuka, Y Oshima. Agr. Biol. Chem. **1968**, *32,* 353
[46] J. J. van Daalen, J. Dams, H. Koopmar, A. Tempel, Recueil **1967**, *86,* 1159
[47] K. Naumann, Synthetic Pyrethroid Insecticides, Structures and Properties, Vol. 4 of Chemistry of Plant Protection, H. Haug, H. Hoffmann (Eds.) Springer, Heidelberg 1990
[48] J. A. Ostrenga, J. Med. Chem. **1969**, *12,* 349
[49] J. K. Seydel, K. J. Scharper, E. Wempe, H. P. Cordes, J. Med. Chem. **1976**, *47,* 3909

[50] C. Hansch, T. Fujita, J. Am. Chem. Soc. **1964**, *86,* 1616
[51] M. Medarde, R. Pelaez-Lamamie de Clairac, J. L. Lopez, D. G. Gravelos, A. San Feliciano, Arch. Pharm. **1995**, *328,* 640
[52] M. M. Hansell, D. D. Ecobichon, Abstr. 13th Annual Meeting Soc. of Toxicol., Washington, 3/1974, p. 57
[53] F. D. Baker, B. Busch, C. Tumasonis, Abstr. 13th Annual Meeting Soc. of Toxicol., Washington 3/1974, p. 59
[54] M. Cueto, C. Brown, Endocrinology **1958**, *62,* 326
[55] K. Findeisen, I. Hamman, B. Hohmeyer DE 3140275, **1981**; Chem. Abstr. **1983**, *99,* 22142u
[56] H. Heidenreich, B. Becker, EP 278337, **1987**; Chem. Abstr. **1988**, *109,* 185491
[57] G. M. Williams, Sci. **1993**, *262,* 15
[58] S. Angsubhakom, Int. J. Cancer **1989**, *43,* 531

Euro Chlor

Euro Chlor is the European federation which represents the producers of chlorine and its primary derivatives. Based in Brussels, Euro Chlor plays a key communications and representation role on behalf of its members, listening and responding to society's concerns about the sustainability of chlorine chemistry.

Euro Chlor
Avenue E. Van Nieuwenhuyse 4, box 2
B-1160 Brussels, Belgium
Tel: +32 2 676 72 11
Fax: +32 2 676 72 41
e-mail: eurochor@cefic.be
www.eurochlor.org