Influence of Chlorine Substituents on Biological Activity of Chemicals

Klaus Naumann

Leverkusen, Agricultural Centre Monheim, Bayer AG

Received April 28th, 1998, respectively March 23rd, 1999

Dedicated to Dr. Pol Bamelis on the Occasion of his 60th Birthday

Keywords: Chlorine, Drug research, Substituent effects, Toxicology

Abstract. A number of well known polychlorinated chemicals are toxicologically and environmentally unsafe. Because of their persistence they are in the focus of public discussions against chlorine chemistry. However, chlorinated organic chemicals in the molecular weight range between 200 and 600 constitute an important and indispensable segment in the arsenal of existing biologically active chemicals used as pharmaceuticals or crop protection agents. Over the course of 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 even crucial for significant activity of a compound derived from nature or chemical synthesis like in the diverse compounds **1** to **12** and **23** to **30**. 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**. Almost all non-reactive chlorinated chemicals and chlorine-free chemicals are devoid of any biological activity at the highest concentration typically used in primary screening tests for discovery of 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 for improvement of 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"), 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, is not justified. Chlorinated compounds are not generally toxic or dangerous. Highly reactive chemicals or polychlorinated compounds can not 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 in the future one of the important tools for probing structure–activity relationships in life science research and as a molecular component in commercialised compounds, in order to provide safer, more selective and more environmentally compatible products with higher activity for medicine and agriculture.

Contents

1. The Background of this Review

People are talking about chlorine. The ecological persistence, negative biological effects and/or other unforeseen negative properties of some organochlorine chemicals that were produced and released in large amounts decades ago are the reason for discussions in recent years at a high political level about a ban [1] on the production and use of element 17 of the periodic table and on

  1436-9966/99/3410X-417 $ 17.50 + .50/0

all organic chemicals containing chlorine. The possibility of carcinogenic and estrogenic effects is of paramount concern. Organochlorines [1]) account for about one sixth of the official list of organic chemicals, for which special safety precautions have to be taken at places of work. 13% of all old (1 050) chemicals traded in amounts larger than 1 000 tonnes are chlorinated organic compounds. 73 of them 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 1 to 6 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 the plant protection agents introduced to the market since 1989, 13% of current pharmaceutical drugs and many technical materials contain a chlorine–carbon bond and 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 however admit that the other side of the coin, risk to humans and nature, was neglected – certainly during the early days. Often this was due to naive 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 the scientific success stories that are subjects for publication in journals and patents. There is, therefore, a strong bias in the information contained in the scientific literature on the effect 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 is perhaps not generally appreciated that it is not really an attractive proposition for an industrial scientist to prepare for scientific publication (and peer review!) results that are neither of practical relevance nor particularly interesting or surprising for fellow experts in other companies. 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 the ongoing discussions outside of the scientific community directed specifically against the use of the element chlorine, this article is therefore focussed on the influence of this element on the biological activity of chemicals. However, chlorine is just one of the substituents which can influence the properties of substances. Each one may have its particular merits in any given case of a biologically active molecule. Industrial chemists are pragmatic people and have no reason for using chlorine when it is not justified.

2. Biological Activity

A "biological activity" is typical for each chemical, and comprises any effect caused in a biological object, not only toxicity. It is depending on the chemical structure. For example, the acute toxicity can be compared in terms of a oral dose which kill 50% of a cohort of the test-animal, usually rat. Tab. 1 shows, that this LD_{50} can extend over a vast dose range of more than ten or more orders of magnitude. This LD_{50} however can be quite influenced by factors up to ten depending on the medium in which the chemical is made bioavailable (solvent, oil, detergent *etc.*). The acute toxicity of a given chemical may also differ by some orders of magnitude amongst related animal species, as was found for TCDD (Tab. 1). These phenomena are also the base for selective herbicides, which may eliminate *e.g.* wild oat weeds in a field of oat. Very high toxicity against weeds is by no means necessarily correlated with toxicity to warm blooded animal. It turned out in the course of the examinations prior to registration to be true only for a very few cases. Moreover, any kind of toxicity is a matter of the applied dose. This basical law of pharmacology and toxicology was discovered by Paracelsus already 450 years ago. He ruled: "dosis facit venenum". It is the base for the possibility to use certain toxic compounds at low non-toxic doses as a medicine. In addition to the structure and dose of a chemical the time frame of action determines its overall effect on a biological object (animal, plant, micro-organism, insect, humans or even a whole biocenosis), Tab. 2.

[1]) 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 publication 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.

Table 1 Scope of acute Toxicity of substances

Substance	Chlorine content	Origin	Minimal Lethal Dose LD_{50} µg/kg p.o. rat
Botulinum toxin	–	bacterium	0,00003
Tetanus toxin	–	bacterium	0,0001
2, 3, 6, 7-Tetrachlorodibenzodioxin TCDD	+	synthetic chemical	1 guinea pig 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
Caffein	–	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
Atrazin	+	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 *e.g.* smell, hormonal effects or acute poisoning. Others are intended to last longer (drugs, pesticides). Some long term effects are undesired, as in the case of environmental pollution or bad working conditions. For toxicological evaluation of a chemical high doses are applied over a long time span.

Tab. 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
Signaling 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 – 10g for the scope of data (see Tab. 2)	$10^{1} – 10^{2}$	seconds – minutes
Narcotic drugs	$10^{1} – 10^{5}$ = 10mg – 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	$10^{4} – 10^{5}$ = 10g – 100g	10^{7}	one month – 24 months
Pesticides	$10^{0} – 10^{2}$ = 1mg – 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 a 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 Scheme 1 the various situations for biological effects caused by chemicals are put into relation to the amount of the compounds and time frame of interaction with a biological system. From there it is evident, that the term "biological activity" is a very complex one and needs always 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 ten up to thousand times greater than the intended commercial administration rate, which is the usual upper limit for the primary testing of a chemical for discovery of intrinsic biological properties in a industrial screening program. In agrochemical terms this means an application rate less than 5.000 ppm or 5 kg/ha for surface application; in pharmaceutical terms a dose less than 10.000 mg/kg body weight or 10^{-4} molar. Higher doses occur only in cases of an accident during manufacture or transport or in cases of deliberate misuse, as in suicide.

Under these 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 high concentrations used. In an attempt to beat these statistical odds, high through-put and combinatorial chemistry technology has been developed in the past few years. Normally, such chemicals are administered only once in the screening test. Repeated administration, which would detect any possible delayed or cumulative toxic effects, is not carried out unless such a compound is a candidate for development.

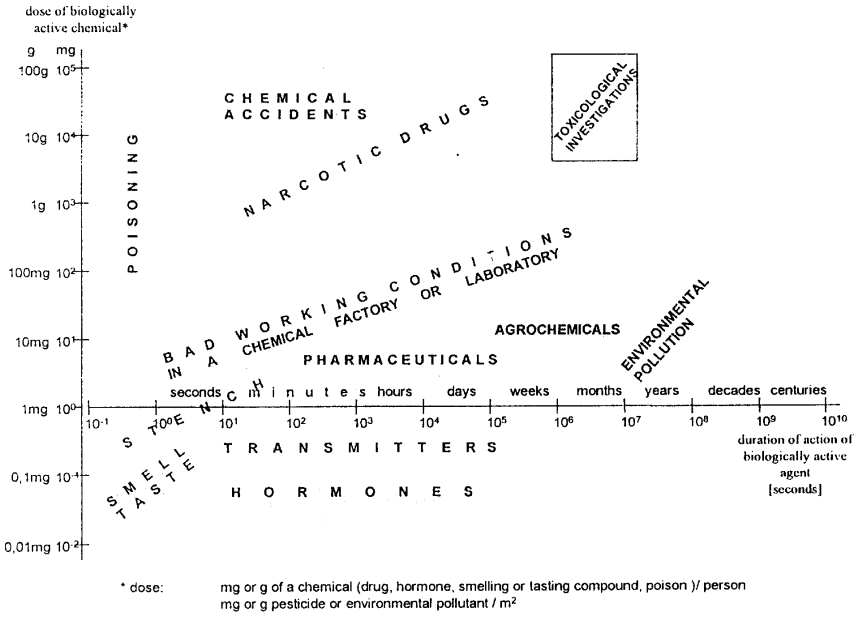
In such cases (the hope of every chemist and biologist involved) the corresponding toxicological data have to be generated in long-term standardised studies according to official guidelines. Using the current state of art it is necessary to prove the safety of the chemical and demonstrate lack of any relevant risk by such *in vivo* experiments. And 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 thousand agrochemicals and three thousand pharmaceuticals, of which about one sixth 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]. However, in the low molecular weight chemicals investigated in that analysis, 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 tethering of the molecule to a bionucleophile such as a DNA base or to an important regulating protein. The modified bionucleophile is then the starting point for mutations or other malfunctions but also for chemotherapia agaist cancer. The same holds



Scheme 1 Biological activity of chemicals and time frame of action

true in principle for any highly electrophilic sp^2 C–Cl bond like the ones found in acid chlorides, activated chloroheterocycles and certain aromatics with a halogen bond activated by electron withdrawing groups. Such chemicals are commonly used in the laboratory as intermediates for nucleophilic displacement reactions in chemical synthesis. 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.

Another important metabolic step is the epoxidation, catalysed by oxygenases, of olefinic or aromatic double bonds to give electrophiles, which are more reactive. 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 give rise to the effects reported by Henschler. However, a chlorine atom at a non-activated aromatic double bond diminishes reactivity, and here the formation of an epoxide and irreversible reactions with bionucleophiles do not take place [3]. This is a very important observation because aromatic moieties play an important role in pharmaceutical drugs and in pesticides.

In polychloroaliphatic systems like CCl_4, nucleophilic displacement is inhibited for electronic and steric reasons. In these 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 radicals and of carbon radicals. These radicals are the cause of further detrimental effects on the function of biologically important proteins or nucleic acids. Such reactivity is well known to chemists and frequently exploited in synthesis for the formation of new C–C bonds by radical addition of polychloroaliphatics 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 -alkenes, chloroquinones, allyl-type chlorides, benzyl chlorides, chloroheterocycles, α-chlorocarbonyl compounds and the like are mostly important highly electrophilic intermediates for chemical synthesis.

3.2. Non-reactive Chemical Interactions with Proteins and Selective Metabolism as the Cause of Specific Biological Activity of Certain Substances

The primary biological properties of drug or pesticide molecules, typically having a molecular weight between 200 and 500 Daltons, originate from strong interaction with mainly proteinous target macromolecules. In most cases this is caused not by an unselective chemical reaction in the classical sense to give new chemical bonds by electron movement along molecular frameworks, as described above for reactive chlorinated molecules, but rather by specific physical supramolecular interactions with a set of amino acid side-chains and peptide bonds. 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 or 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. The occurrence, structure and function of such functional proteins differ in by fare the most cases 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.

Bioactive compounds, when applied as single dose or by short-term exposure, are usually rapidly metabolised in the human body, and thus rendered harmless and excreted as biologically unavailable derivatives (this can be demonstrated with radioactively labeled compounds). This metabolic power is different in plant, even in related plant species, micro-organism, insects and rats. Together with different protein binding niches this contributes very much to the occurrence of very selectively acting compounds, like certain neurotoxic compounds which interfere only with the neurosystem of insects but not with the nerves of warm blooded animal. Imidachloprid **90** (Tab. 6) is one example.

In a few cases however, due to low chemical reactivity, resistance to metabolisation and high ecological persistence combined with high lipophilicity, certain chemicals such as the polychlorinated ones are causing the infamous problems that are the reasons for the element chlorine becoming involved in politics.

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

4.1. Physicochemical Properties of the Chlorine Substituent

Four parameters of chlorine, relevant for its toxicology due to its chemical reactivity, have recently been specified [2, 3].

1) High electrophilicity at the carbon connected to a chlorine atom
2) Electronic effects caused by chlorine increase electrophilic reactivity at more remote carbon atoms
3) Low bond energy in alkyl chlorides gives high reactivity
4) Increase in lipophilicity of the molecule.

In addition to these properties, which are also connected to parameters mentioned below, the C-bonded chlo-

rine has an oxidation potential. This enables certain micro-organisms to cleave the C–Cl bond in a reductive manner under anaerobic conditions, as in naturally produced organochlorines in sediments [4]. For non-bonding interaction of a drug or pesticide with proteins, the chlorine atom decorating a larger molecule as a substituent provides a number of interesting features:

– Hammett constant of the chlorine substituent on a phenyl ring
– A space-filling volume provided by the van der Waals-radius of the chlorine atom
– lipophilicity of the carbon-chlorine fragment
– electrostatic force-field radius of Cl, relevant for interactions with other local electrostatic fields of a protein
– high electronegativity of the chlorine atom
– polarisation of the aromatic system attached to the chlorine
– dipole moment of the carbon-chlorine bond
– free electron pairs at chlorine as hydrogen-bond bridgeheads
– London dispersion forces of the outer electrons around chlorine for non-bonding interactions with other electron pairs.

These give rise to the 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 residues surrounding the position of the chlorine atom in the binding pocket of the protein. This in turn may cause a tighter interaction or loosening of the contacts to the amino acids there or in other parts of the molecule. Either one may affect the function of the target protein and hence influence the biological activity. In other cases a chlorine substituent may have no specific effect on the primary biological properties of the molecule to which it is attached.

The most important effect of a non-reactive chlorine atom on the biological activity of many compounds comes from chlorine as a substituent on an aromatic, heteroaromatic or olefinic moiety. The presence of this chlorine causes

– an increase in lipophilicity
– non-bonding interaction with protein groups in the binding site
– fixation of an active molecular conformation necessary for interaction with a protein.
– direct increase in electrophilic reactivity of proximate or remote parts of the molecule due to its electron-withdrawing effects caused by its electronegativity, but without involvement of the C–Cl bond
– direct increase in the acidity of a not so distant NH or OH bonds present in the molecule, due to the electron-withdrawing properties of chlorine.
– diminution of basicity of neighbouring nitrogen atoms
– prevention of metabolic hydroxylation at that position.

4.2. Chlorine Substituents and Biological Activity

It is an old observation that a chlorine substituent may cause a significant increase in activity. This used to be a reason for scientists to keep a special look-out for chlorinated compounds from fermentation broths.

However, many halogenated compounds from nature were not first identified *via* their biological activity. Rather they were discovered during structural elucidation of unknown compounds isolated from natural sources.

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 of these commercial chemicals and lack of thorough studies on inactive or less active congeners (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, cannot be 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. If a positive in-vitro result in the Ames test for mutagenicity towards some bacteria always had to be taken into account, as is the case during the rigorous investigation of a commercial bioactive compound, then 50% of the natural components of our daily food would have to be discarded as carcinogenic [8]. Moreover, 50% of the isolated natural food components fed at maximum tol-

erated doses to test animals turned out to show 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, culled from the literature as well as unpublished titbits from every-day screening results in an industrial laboratory, 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 is it for humans and the environment and the fewer the resources that are consumed in its production. The more selective a compound is, the smaller the chance of untoward side-effects.

4.3. Chlorine Substituents as an Essential Feature in Some Biologically Active Compounds

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

The old insecticidal polychloro compounds, with their widely discussed negative properties, can be named here. But even in less highly chlorinated or monochlorinated compounds there are many examples where the chlorine-free analogue is practically devoid of biological activity (Tab. 3).

In the case of DDT **74**, Tab. 6, the presence of the CCl_3 moiety is essential. 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 not just the mere presence of a chlorine in the molecule is necessary; it has to be in the right position. This principle was discovered long ago by Nature with its own chlorinated biologically active ingredients (Scheme 2). 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], neopyrollomycin **8** [15] and astin A **9** [16]. In case of the natural azaphilons **10** [17] the chlorine exerts its enhancing effect with regard to binding to the endothellin receptor of rats and rabbits in quantitatively quite a different way. The chlorinated hydroxyketones **11** (Scheme 2) are important signaling compounds in slime moulds. Switching off this signal is accomplished by enzymatic dechlorination [18].

The two chlorine substituents in vancomycin (**2**) are needed to induce a specific conformation required for inhibition of 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 2), ecologically a very important chlorinated trace compound, 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 3 shows a selection of examples where the chlorine-free compounds are much less active at 0.1% concentration (1 000 ppm) in the test medium. The increase in activity following introduction of chlorine substituents is associated here 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** (Scheme 3) [22], the trichloro analogue of saccharose, can be explained by this way. In addition, due to the electronegativity of chlorine, an acidification of neighbouring NH or OH bonds may occur, giving rise to an anionic species, which, when combined with high lipophilicity, is capable of shuttling protons across the cell membrane. This causes a collapse of proton gradient across the membrane and an end to energy production in the cell by uncoupling of phosphorylation [23].

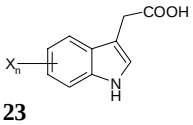
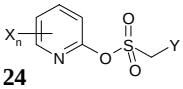
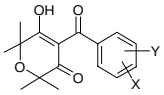
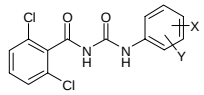
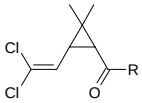
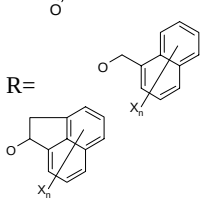
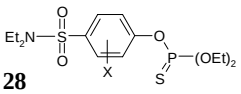
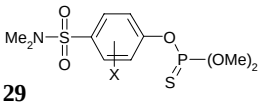
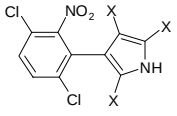
In case of chlorophenols **14**, high adsorption to albumin is correlated with high adsorption to mitochondrial proteins and inhibition of phosphorylation; the inhibition factor increases by a factor of 100 with increasing chlorine content [24] from Cl_1 to Cl_5.

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

The inhibition of 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 the chlorinated benzoquinones **18**, the increase in activity going from parent quinone to tetrachloroquinone is much less pronounced [25]. Salicylic acid, an important signaling compound in plants and regulator of oxidative events in mammals (pharmacologically important in humans as its *O*-acetyl derivative) is also an inducer of genes in plants, when taken up by the roots from a 10^{-5}M solu-

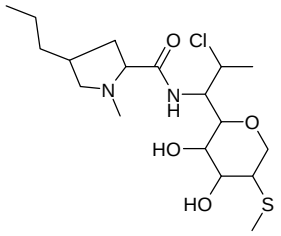
Tab. 3 Chlorine substituents as modulators of biological activity (X = H, Cl)

Compounds	Biol. Effect	Structure-Activity-Relation Substituents X,Y	Ref.
 **23**	X = H, Cl Natural plant growths hormons 4-Cl: "death hormon?"	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]
 R=  **27**	Insecticide	Cl_2 > H H > Cl_2	[40]
 **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]

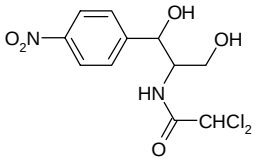
tion. Introduction of a 5-chloro substituent **19** renders salicylic acid ten times more active [28]. However, the corresponding *O*-methyl and *O*-acetyl derivatives are both inactive. This indicates a physiologically important increase in acidity of the free phenolic or carboxylic group, combined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

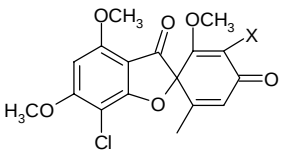
However, a chlorine substituent in the meta-position causes a fourfold increase in inhibition [30], demonstrating that here a third effect is operating in addition to the two aforementioned ones (increases in acidity and



1 Clindamycin

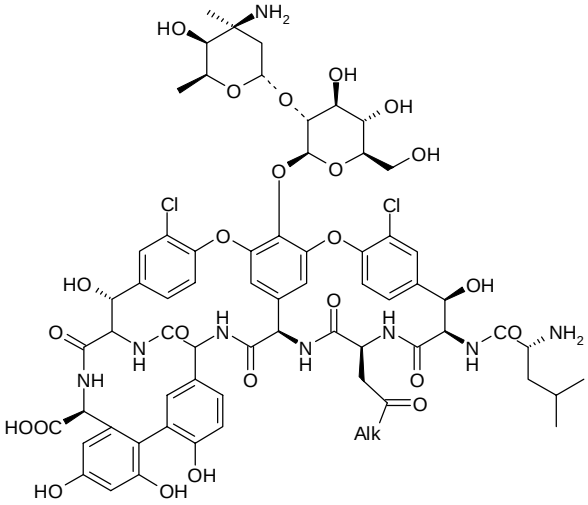


3 Chloramphenicol

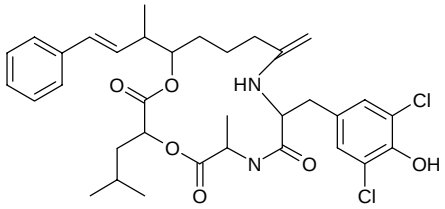


X = Cl / H

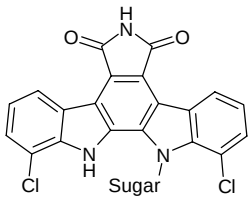
4 Griseofulvin



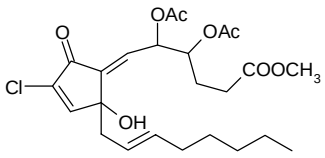
2 Vancomycin



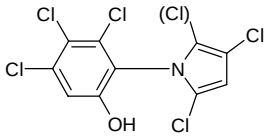
5 Cryptophycin



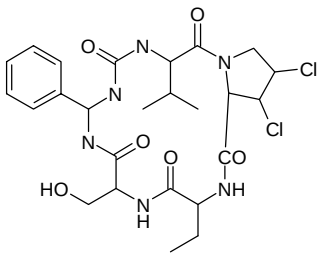
6 Rebeccamycin



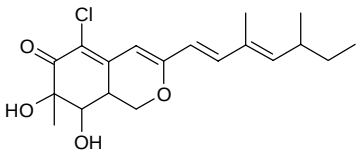
7 Clavulon



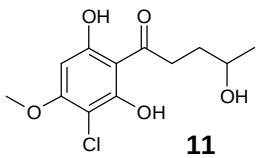
8 Neopyrolomycin



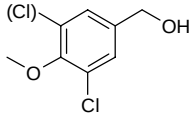
9 Astin A



10 Azaphilon

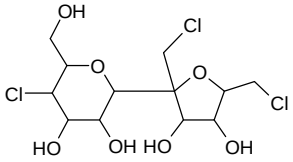


11

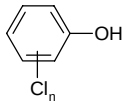


12

Scheme 2 Naturally occuring chlorinated biologically active compounds, where chlorine is necessary for activity

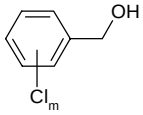


13 Sucralose



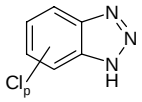
n = 0 - 5

14



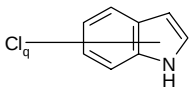
m = 0 - 5

15



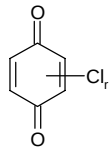
p = 0 - 4

16



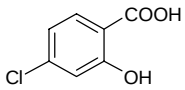
q = 0 - 5

17

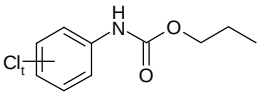


r = 0 - 4

18

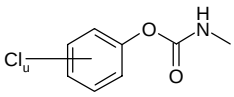


19



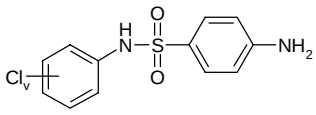
t = 0 - 2

20



u = 0 - 2

21



v = 0 - 2

22

Scheme 3 Chlorine substituents as enhancer of biological activity

lipophilicity), namely the structural factor of chlorine substitution. The same effect is observed in the case of bactericidal phenylsulfanilides **22** [30].

Another structural factor, steric interference at the ortho-position of chlorophenols renders the ortho-chlorophenol less toxic to waterfleas 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

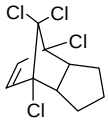
As shown above, chlorine may be essential for a compound to have any biological activity. In other examples it causes a strong enhancement of the weak intrinsic biological activity of the parent molecule. Most of the commercial chlorine-containing pharmaceuticals and crop protection agents, either isolated from natural sources or invented in synthesis laboratories, have been selected for this reason. 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 have 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 a heterocyclic moiety, from fluorine to a sulfamoyl group and so on, and encompasses hydrophilic amino and hydroxy groups, piperazinyl, morpholinyl and other basic moieties.

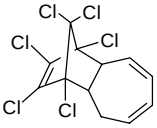
The compounds shown in Table 3 have an optimal number and/or substitution pattern of chlorine atoms for best activity. It is not just the presence of a chlorine atom that causes biological activity. The whole molecule is involved, whereby some structural features are more or less variable and others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

The relative contributions of each substituent to the measured activity can be determined by an analysis of quantitative structure–activity relationships (QSAR), derived from a series of analogues,.

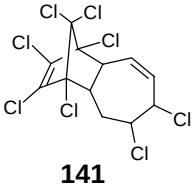
An interesting example demonstrates the structural factor by which replacement of a hydrogen atom by chlo-



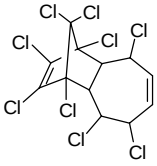
139



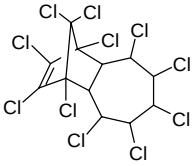
140



141



142

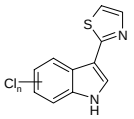
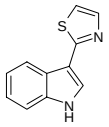
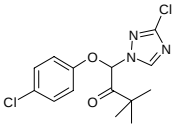
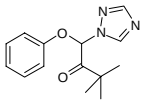
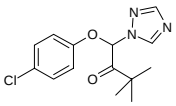
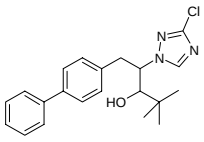
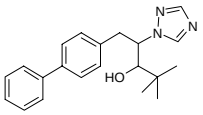
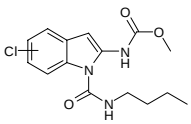
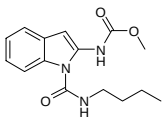
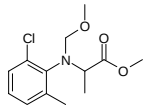
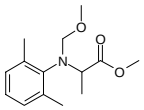
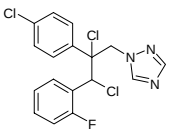
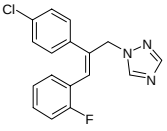
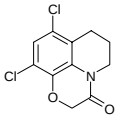
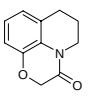
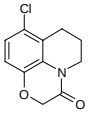


143

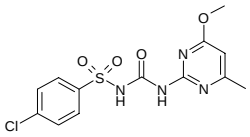
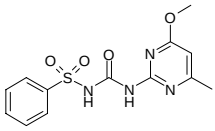
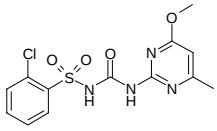
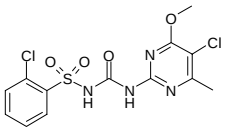
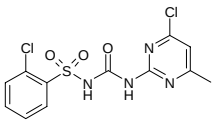
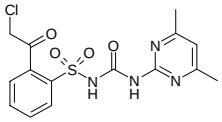
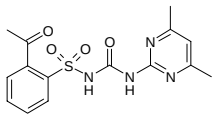
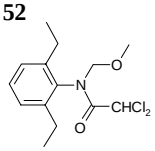
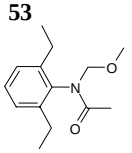
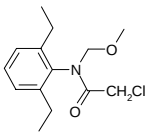
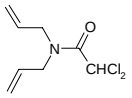
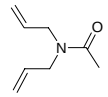
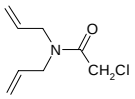
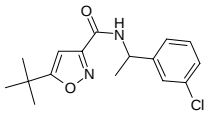
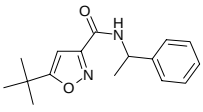
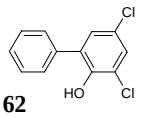
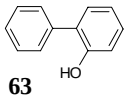
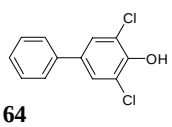
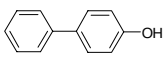
Scheme 4 Diminishing insecticidal activity with increasing chlorine content in Chlordane analogues

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

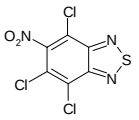
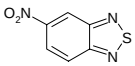
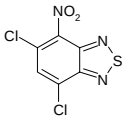
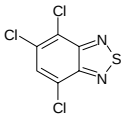
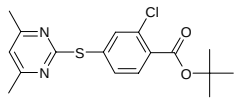
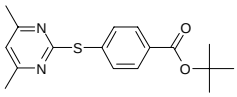
Tab. 4 Shift in fungicidal activity by chlorination

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

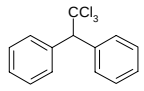
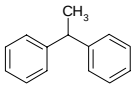
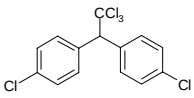
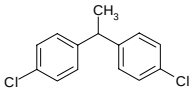
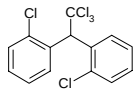
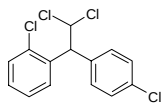
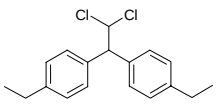
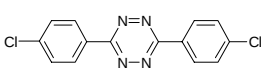
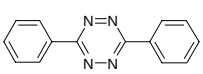
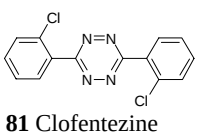
Tab. 5 Shift in herbicidal activity by different chlorine substitution pattern in some herbicide classes

Less Active or Inactive Chlorinated Derivatives	Activity	Parent Compound	Activity	More Active Chlorinated Derivatives	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]

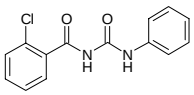
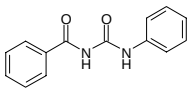
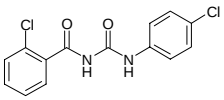
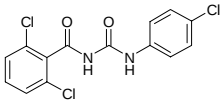
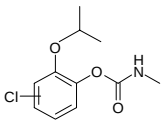
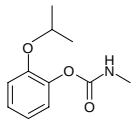
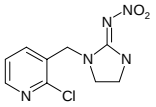
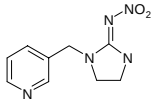
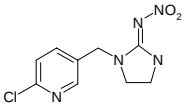
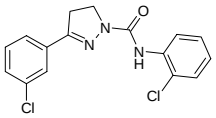
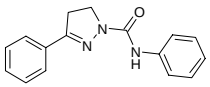
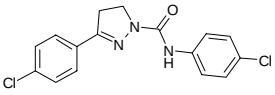
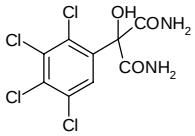
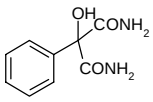
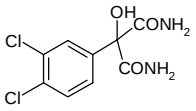
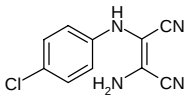
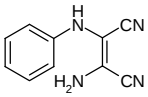
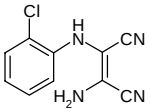
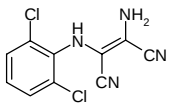
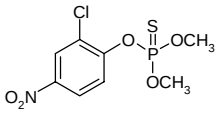
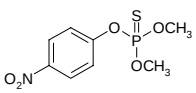
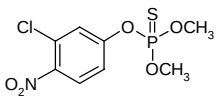
Tab. 5 (continued)

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

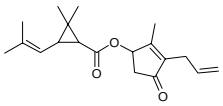
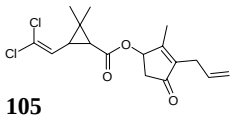
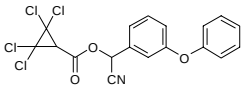
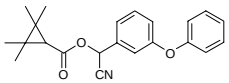
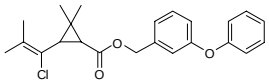
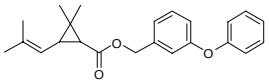
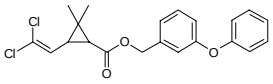
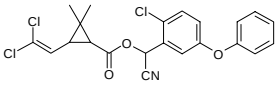
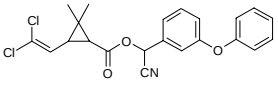
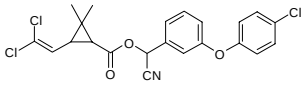
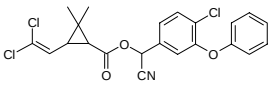
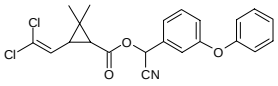
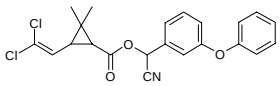
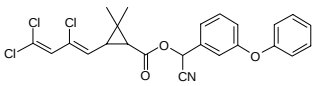
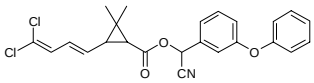
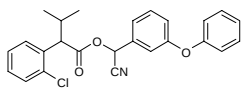
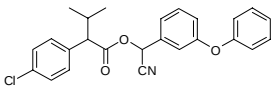
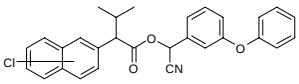
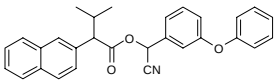
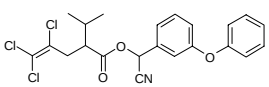
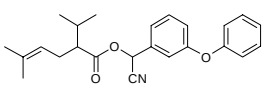
Tab. 6 Shift in insecticidal activity by different chlorine substitution pattern in various classes of insecticides

Less Active or Inactive Chlorinated Derivatives	Activity	Parent Compound	Activity	More Active Chlorinated Derivatives	Activity	Ref.
	–					
72		**73**		**74** DDT	++++	[44]
	–					
75						
	–					
76						
	– Antitumor activity		++			[54]
77 Mitotane		**78** Pertane				
	–		–		+++	[44]
79		**80**		**81** Clofentezine		

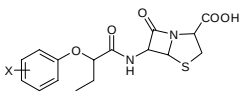
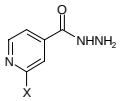
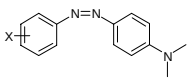
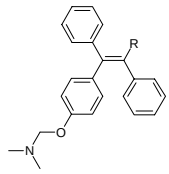
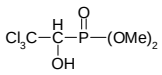
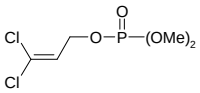
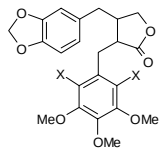
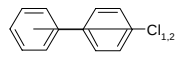
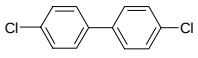
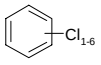
Tab. 6 (continued)

Less Active or Inactive Chlorinated Derivatives	Activity	Parent Compound	Activity	More Active Chlorinated Derivatives	Activity	Ref.
 **82**	–	 **83**	–	 **84**	++	[44]
 **85**	–					
 **86**	–	 **87** Propoxur	+++			[44]
 **88**	(+)	 **89**	+	 **90** Imidachloprid	++++	[44]
 **91**	–	 **92**	–	 **93**	++	[44]
 **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					

Tab. 7 Influence of chlorine substituents on insecticical 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* α *R* enantiomer	–	 **116** *R trans* α *S* enantiomer	+++++		
 **117**	+	 **118**	+++		
 **119**	(+)	 **120**	+++		
 **121**	–	 **122**	++		
 **123**	–	 **124**	+		

Tab. 8 Diminuition 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	λmax 10^{-6}	2Cl	λmax $4 \cdot 10^{-6}$	[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]
			 **130**	not carcinogenic	[44]
 **131**	antitumoral		$2,6Cl_2$	less active	[51]
 **132**	kidney damage in rats		higher chlorinated congeners	less damage	[52]
 **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

Contrary to the hopes of the researchers in the course of a programme for optimisation of a lead structure, it often turns out that 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 interest.

However, it is not so easy to retrieve such information from the records because the chemists involved are only looking for an improvement in biological activity and/or toxicological or environmental properties. Failures are usually consigned to oblivion. Information on chlorine as a diminisher of biological activity would not be particularly sensational for these experts (it is actually not an uncommon occurrence) and regarded by them as unworthy of publication. Since such information is very rarely found in the literature, it is at best more of a collector's item. The author has managed to compile a number of examples, shown in Tables 4–8, drawn from his own results gathered during his 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 and 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 4) [33]. The author has a number of examples at hand from his own involvement in pyrethroid research showing how a number of chlorine substitutions at specific positions of an active molecule can modulate insecticidal activity in both ways, enhancement and diminution (Tab. 7), besides having no effect.

5. Access to More Information

In principle this type of information on the influence of chlorine atoms on biological activity can be retrieved from all 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. It is hoped that more collectors of such – mostly – raw screening data will exchange this information at future conferences or submit it for publication.

In this respect this review is intended to initiate a scientific discussion about sharing the burden of information with the public even on matters that are, in the strict scientific sense, not very exciting to the experts in possession of such empirical data when measured against the standard of scientific papers published by their university colleagues. However, it would be a 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, as some recent scientifically nonsensical statements about chlorine demonstrate.

6. Summary and Outlook

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

Group 1) Polychloro-organics and alkylating chemicals of lower carbon atom number and/or high chlorine content. Chlorine, due to the electrophilic or radical reactivity of the C–Cl bond in such chemicals or their metabolites, or conversely their non-reactivity leading to persistence, is responsible for the biological long-term effects (toxicity) seen under continuous exposure. These effects increase with the degree of chlorination. To this group belong the 120 mostly low molecular weight polychloro compounds comprising solvents, reactive chemicals, chemical intermediates and other chemicals elaborated in detail in Henschler's book on 'Toxicity of Organochlorines' [3]. Only a few of them were originally selected for uses based on their biological properties.

Group 2) Monochloro- or oligochloro-organics with a high number of carbon atoms (mol. weight 250–1 200). The chlorine substituent in a given naturally occurring or synthetic chemical is crucial for the observed biological activity.
Examples: Some natural antibiotics and some pesticides.

Group 3) Monochloro- or oligochloro-organics with a high number of carbon atoms (mol. weight 200–600). Chlorine substituents at specific positions increase an intrinsic lower activity several fold.
Examples: Almost all compounds belonging to groups 2–3 practically useful as drugs or pesticides, are of 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. The registration authorities, applying the highest available scientific standards for evaluation of pesticides and drugs, had approved about 140 chlorinated pesticides [34] and about 120 chlorinated pharmaceutical drugs in Germany as of 1992 [35]. About 330 chlorinated pharmaceutical drugs are listed in the latest Merck Index [36].

These figures do not include reactive cytostatics, disinfectants and chloride salts. This is proof that such com-

pounds are neither carcinogenic nor toxic at levels relevant to the extended scope of practical use.

Group 4) Monochloro- or oligochloro-organics with a high number of carbon atoms and low chlorine content (mol. 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: Unsuccessful, usually unpublished results from industrial drug and pesticide research. The about 70 cases presented in this review alone from one institution certainly represents only a small part of what is known. An in-depth literature survey would yield many more examples.

Group 5) Chlorine-containing chemicals (mol. weight 200–800) with a high diversity of structures and wide range of physicochemical properties that show no activity in primary screens involving many *in-vitro* and *in-vivo* tests for biological activity, including toxicologically relevant target proteins.
Examples: 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 teaching that chlorine renders a compound *per se* more toxic or more active. 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 not safe enough for use as a drug or pesticide are discarded, whether they contain chlorine or not.

However, each chlorinated test compound in the process of lead structure optimisation towards a better drug or pesticide 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 possibly most interesting biological activity may have been prematurely classified as unfruitful because these structures were not probed at the proper positions with the right substituents, of which chlorine is but one.

I thank K. H. Büchel for providing his collection of old structure/activity data from the 1960s/1970s. The help of some of my fellow chemists in Central Research Laboratory and Crop Protection Research of Bayer AG in providing some data is appreciated. I thank G. Holmwood for help in preparing the manuscript.

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*, 1056; R. Pairet, S. K. Wrigley, J. Chettland, E. E. Reynolds, M. A. Hayes, J. Holloway, A. M. Ainsworth, 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. Smith, 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 Organohalogens (Eds. A. Grimvall, E. W. B. de Leer), Kluwer Academic Publishers Dortrecht **1995**, p. 227
[28] C. Lechelt-Kunz, Central Res. 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 Index, **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. Sakamoto, Y. Nishizawa, Agr. Biol. Chem. **1970**, *34*, 1697
[42] S. Neidleman, 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

Address for correspondence:
Dr. Klaus Naumann
Bayer AG
Landwirtschaftszentrum Monheim
Chemische Forschung, Geb. 6550
D-51368 Leverkusen
Fax: Internat. code (0)2173 38-4107
e-Mail: klaus.naumann.kn@bayer-ag.de