Session 1

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Chairman	Mr J NORTH
Speaker	Professor J HILLMAN
Session Organiser	Professor D ATKINSON

BIO-ENGINEERING - INTELLECT, ENTERPRISE AND OPPORTUNITY

J.R. HILLMAN
Scottish Crop Research Institute, Invergowrie, DUNDEE, DD2 5DA

ABSTRACT

In this article I explore the benefits to mankind of various kinds of technology used to introduce new characteristics in living organisms, and consider the issues arising from the creation of new life forms. Bioengineering in its broadest definition includes engineering required for various methods to synthesise animal, plant and microbial products, and also includes devices to assist in the functioning of the human body. Recombinant DNA technology - genetic engineering - is the ultimate engineering, however, allowing mankind both to explore the very processes of life and to exploit the intellectual products. The subject matter encompasses a vast literature, replete with jargon and complex concepts. It is also one of the most rapidly advancing, innovative areas of science, worthy of substantial investment by private industry and by government alike. We live in the age of the biologist.

INTRODUCTION

Last year, Professor C. R. W. Spedding presented the nineteenth Bawden Lecture, choosing as his topic the role and impact of technology, legislation and public opinion on modern agriculture. He noted that effective demand has the dominant impact on modern agriculture, and that technology is essentially an enabling procedure constrained by legislation, need and economics.

This paper focuses on technology of a special kind - variously referred to as bioengineering, genetic engineering, recombinant (r)DNA technology, genetic manipulation, genetic modification, depending on the sort of image that is meant to be conveyed. When applied to genetics, "engineering" has a strongly negative image in the minds of the public, many politicians and pressure groups; it indicates unnatural procedures with potentially calamitous risk. In my view, bureaucratic obfuscation, euphemisms, undue sensitivity, secrecy and dithering are no substitute for an open clinical approach to risk assessment, and recognition of the sheer brilliance and potential of the technology. It cannot be de-invented; it must be exploited responsibly. The need is pressing.

Genetic engineering currently has its greatest applications in pharmaceuticals and drug design. Studies on catalytic and other antibodies, human gene therapy, the Human Genome Project and drugs whilst being anthropocentric nonetheless mutually interact with related studies on plants, fungi and microorganisms to advance knowledge in the whole of genetic engineering. I shall concentrate on crop plants, the underlying theme of this Conference, avoiding as much as possible specialist vocabulary.

THE NEED

Agriculture in Developed Countries

From the perspective of most members of the public in the western world, agriculture is not viewed as a priority topic. In fact, its importance is understated. Most references to economic analyses in agriculture and horticulture relate simply to gross traded values of commodities, hectares grown, yield estimates, major categories of land use, imprecise crop and livestock categories, unit prices, direct employment and percentage origin of Gross Domestic Product. Data relating to (i) processing values for the food and non-food sectors; (ii) development and maintenance of rural, industrial and marketing infrastructures; (iii) amenity and tourism; (iv) influence of subsidies; (v) costs of import substitution; (vi) assessments of trends in and prospects for exports and trading; (vii) relative social values to the national and local economies; (viii) cultivar performance and market share; and (ix) indirect employment, are usually ignored because they are often unreliable, incomplete, out of date, anecdotal, disputed or subject to commercial secrecy. International comparisons are made especially difficult by variations in the methods and dates of sampling, unspecified types of analyses and fluctuating currency exchange values. As a general point, it is germane to note that arable and horticultural crops produced in the UK had an annual value of £6,527 billion in 1990 (1.4% of Gross Domestic Product) before industrial processing and value-added contributions. During the past three decades there have been dramatic, research-driven, improvements in commodity processing, crop productivity and efficiency throughout the world.

Within Europe, public and political opinions of the effects of the Common Agricultural Policy of the European Communities tend to relate to unwelcome changes to the countryside, expensive food surpluses/stockpiles, subsidies, quotas, set-aside land, extensification, restrictions on pesticides and nitrogenous fertilizers, undignified animal welfare, and reappraisals of R&D priorities. All in all, opinions are not favourable towards agriculture, although the products are a basic need and civilisation is agriculture-dependent.

Population Pressures

A billion extra people are projected to be added over the next ten years to the world population which presently exceeds 5.4 billion (see annual reports from the World Commission in Environment and Development; FAO, World Resources Institute and Worldwatch Institute). Inordinate strains will be placed on the less-developed countries for food, water, shelter, fuel, education and welfare. Large parts of Africa, especially, face dismal prospects. Access to the media and advanced medicine, though, will ensure that all citizens will demand improving quality of life regardless of local economic situations. Low-grade grazing systems coupled to poor, unsustainable agricultural systems will inexorably lead to the acceleration of deforestation, soil erosion, desertification and the rapid loss of natural and managed ecosystems, destroying genetic and environmental diversity. Social instability, emigration and trade disruption seem inevitable for the poorer of the less-developed countries.

Largely as a result of major if unsung technological successes in the recent past, especially in plant breeding and pathology, agriculture and the related life sciences are universally assumed to be able to adapt without major investment to meet the challenges of population growth. At the same

time, it is expected that agriculture should not adversely affect the natural flora and fauna, nor exacerbate any potentially undesirable effects ("climate change") of the changing gaseous composition of the atmosphere since the advent of the Industrial Revolution.

It seems remarkable that there is so little publicity given to the loss of cultivated land throughout the world. Soil erosion, pollution, buildings, roads, airports, and recreation facilities account for the main loss of productive land. Modern monocultural agricultural systems can cause problems, e.g. use of xenobiotics, soil compaction and erosion, salinity effects and changes in the soil flora and fauna, but traditional methods (e.g. slash-and-burn, uncontrolled grazing) can be even more erosive without even being productive. All too frequently, third-world agriculture can incorporate many of the bad practices of high-input agriculture now being phased out in the western world. About 85% of the growth in population occurs in developing countries where the numbers of malnourished people have increased by 35% since 1980. In the tropical zones, the area of cultivated land per capita has declined from 0.28 ha in 1971 to 0.22 ha in 1986; this figure masks urbanisation, fragmentation of farms, and expansion of cultivation into virgin lands unsuitable for arable farming in the medium term.

Given that the area of land under cultivation is a limited resource and difficult to increase without massive migrations of people and devastation of forests, that pests and diseases have a phenomenal ability to circumvent control measures, and that research and development demand long-term commitment, the global picture is far from bright until the demand of the world population matches sustainable resources.

One possible or probable scenario is that the industrially under-developed - or low income - world will become the major source of manufactured goods, effectively reversing the trend in trade established since the Industrial Revolution (Carruthers, 1993). The economies of most of the countries of the Pacific rim are buoyant, and in Asia there are several countries with sophisticated, urbanised workforces able to operate efficiently and compliantly with relatively low incomes. Scientific intercommunication, multinational trading, and improving education in the low-income world ensure that invention, intellectual property and service industries will not be the preserve of the present developed world. Moreover, agriculture in developing countries is no longer regarded as the engine of economic growth - witness the pressures on the Consultative Group on International Agricultural Research. Thus it is likely that most of the world's food production would take place in the temperate zones. Whether or not there would be the means to pay for the food is a moot point.

One global feature is the growing divide between stable or expanding urban populations and their rural counterparts. By way of example, in the UK 91.5% of the population is urban, with relatively low mobility, a birth rate per 1,000 population of half the world average, and a population doubling time in excess of 100 years. The overall population density is high (235 persons km^{-2}) revealing the extent of crowding in the urban area. No wonder misunderstanding of the rural economy is becoming so pronounced.

Conventional management of terrestrial and aquatic resources will not meet future demands. Over 90% of the world's population depend on just 15 plant and 7 animal species for food (Hillman, 1992); a tiny genetic reservoir to combat the ravages and vagaries of pests, diseases and inclement conditions. To this must be added the fact that as the only animal to cook

food and thereby broaden the range of acceptable food species and types, mankind is faced in the arid and semi-arid regions of the developing world with a shortage of fuel for cooking.

Woody perennial species present one of the stiffest challenges for crop management. New initiatives are desperately needed for breeding, selection, propagation and health of trees and shrubs.

Plant Breeding

Central to the ability of the bulk of the population to move from food cultivation and harvesting to engage in social and technological advancement is the provision of improved crop plants throughout the ages. Plant breeders have always been involved in genetic engineering. Characters including yield performance, resistance or tolerance to pests and diseases, quality components, uniformity and lack of prolonged dormancy periods represent the main selection criteria. Together with advances in automation, storage and processing there is a complacent view that plant breeding will perpetually answer basic nutritional needs for burgeoning populations. This is unacceptable.

Plant breeding programmes are protracted, expensive and are rarely allowed to proceed without interference. Basic to the needs of such programmes is access to genetic resources for parental material. Unfortunately, there has been severe attrition of genetic diversity by losses of diverse wild habitats, traditional farming area, valuable collections and obsolete landraces. Breeders need to screen vast numbers of clones over many years, carry out regional trials, multiply stocks, access statutory trials and be involved in marketing. Other problems faced include imprecise predictions of genotype by environmental interactions, incompatibility systems between and within species affecting the ability to cross-breed, juvenility or ripeness-to-flower phases, seasonal growth patterns, changing disease virulence patterns and disease vector distributions, and complex breeding objectives involving polygenic characters. Conventional plant breeding is well-established but needs to be supplemented by bioengineering technology to allow access to new sources of genetic variability, to speed up the process, to unravel the complexities of genomes (genetic constitutions), to understand the processes involved in breeding, and to improve the prediction of performance of products arising from the breeding programmes.

ENGINEERING PLANTS

Modern technologies are reducing the reliance on a combination of serendipity and bulk selections for plant breeding and food processing qualities.

Modern biotechnology has its beginnings with the well-known early studies on deoxyribonucleic acid (DNA - actually a base!) molecules of which act as the carriers of genetic information. In 1973 recombinant DNA techniques were discovered at Stanford University in California. Shortly thereafter, animal hybridomas were created in the Laboratory of Molecular Biology, Cambridge, a discovery which initiated the monoclonal antibody diagnostics industry. During the 1980s, the inherent similarity of the genetic language in the major groups of organisms was demonstrated by the insertion of genes or sequences into the DNA of recipient (transgenic, genetically manipulated or modified, or recombinant) organisms and sub-cellular entities containing nucleic acid.

These insertions (constructs) lead to the induction of new traits in the genetically modified organisms (GMOs).

In this period, the speciality of protein engineering came to the fore, with an aim of producing customised, biologically active (eg enzymes, antibodies) and structural (eg collagen) proteins for a wide range of purposes. Proteins are genetically coded amino-acid polymers with a molecular shape that helps determine function. Studies in protein engineering have been particularly facilitated by site-directed mutagenesis of the genetic code.

Both DNA and ribonucleic acid (RNA) consist of long sequences of nucleotide bases that are attached to a backbone of alternating sugar and phosphate groups. It is the sequence of bases that constitutes the genetic code. A gene is a portion of nucleic acid that carries the code for synthesising a specific protein or part of a protein. Each nucleic acid molecule is comprised of a linear series of genes. Between and within genes (the coding sequences or exons), though, there tend to be the so-called introns, or intervening sequences of nucleotide bases that apparently do not code for proteins. Functional RNA molecules have the introns removed or "spliced out" by cutting out and joining (ligating) the cut ends together.

DNA acts as the blueprint whereas RNA of different types act in a wide range of functions, including the role of messenger for conveying the blueprint code to the various sites in the cell where protein synthesis takes place.

Splicing and Ribozymes

Splicing is one of the key control points for cell metabolism, development and differentiation generally (the "splice of life") and the mechanisms of intron recognition and splice-site selection are crucial to understanding regulated gene expression, especially in transgenic plants. Certain RNA molecules have the ability to act as catalysts - the ribozymes - which have led to the application of the so-called gene shears for selectively cleaving RNA molecules without the presence of any protein; they may also be used to inactivate or destroy RNA viruses.

Antisense Technology

Gene expression in living organisms can be prevented by synthesising relatively short RNA or DNA molecules (oligonucleotide primers or oligomers) which bind specifically and selectively to complementary sequences on the target RNA or DNA molecules, switching off genes such as those controlling ethene (ethylene) biosynthesis. Both ribozymes and antisense technology can be harnessed to combat viruses and control developmental functions in plants.

DNA Fingerprinting or Profiling

When DNA is cleaved by highly specific restriction enzymes the lengths of the resultant fragments produce exceptionally reproducible patterns (restriction fragment length polymorphisms. RFLP) in electrophoretic separations. The patterns are inherited and can be used for diagnostic purposes. RFLPs (Bottstein et al. 1980) are used for tagging genes with tightly linked markers for selection in plant breeding programmes. They are also used for map-based gene cloning, assessment of genetic variability. and also for comparative genome mapping to study relationships between organisms. Locating genes with respect to DNA markers on an RFLP-based map provides a

starting point for cloning genes by "chromosome walking" down overlapping large pieces of the chromosome from the RFLP tag to the gene of interest.

Polymerase Chain Reaction (PCR)

A major advance has been the ability to produce simply, consistently, automatically and cheaply, identical copies of specific DNA sequences. In essence, and there are many variants, PCR involves a series of copying cycles in which double-stranded DNA fragments are firstly denatured to provide templates for the "annealing" or binding by base pairing of two synthetic primers (short DNA molecules of known sequence that flank either side of the target region) to complementary sequences on the DNA template strands. Thereafter, the bound primers are elongated enzymically by the addition of nucleotides from the reaction mixture. The resultant new DNA strands are complementary to the template strands. One PCR cycle duplicates one DNA fragment and hence produces two copies. This amplification system is employed to produce adequate amounts of single or multiple genes for both fundamental and applied studies. PCR has also been used for demonstrating, mainly in animal genome mapping, high levels of polymorphism in the repeat number for simple sequence tandem repeats or microsatellites which are potentially ideal genetic markers.

The DNA Approach

From the foregoing, a basic shift in philosophy has taken place in the past few decades, from the inference of the genotype from a study of phenotype morphology as was once carried out by conventional plant breeders, to a direct analysis of DNA sequence information i.e. a change in emphasis from Mendelian to genomic genetics.

Selectable Marker Genes

The incorporation of dominant selectable marker genes (marker genes or markers) together with the DNA construct aids in the identification and selection of transformed cells from a background of non-transformed cells. Marker genes also assist in confirming the identify of transgenic plants for legal purposes. Marker genes may encode a protein or enzyme that modifies a toxic substance to render it harmless, thereby allowing the transgenic cell to grow in the presence of the toxic substance. Other encoded proteins may react with compounds to produce chromogenic compounds or emit light. Yet others enable the degradation of organic compounds, the utilisation of sugars, or the conversion of heavy metal components into their metallic form. Some are even silent, producing specific, amplifiable DNA fragments e.g. palindromic sequences.

Plant pathogenic microorganisms and viruses have been a major source of DNA fragments for constructs which contain the marker gene. Bacterial transfer processes (e.g. Agrobacterium, Escherichia), chemically induced gene transfer, electroporation, liposomes, injection and particle bombardment, imbibition and incubation are the most common procedures for introducing the constructs.

In addition to normal biochemical assays, the marker gene protein or enzyme can be isolated and detected by Western blotting, in which total proteins are extracted, separated and reacted with antibodies with specificity towards the gene product protein. The specific complex formed between the

antibody and the protein product can then be detected using as little as 5 x 10^{-9} g by a secondary reaction directed at the complexed antibody.

Molecular assays, such as Southern or DNA blotting, detect the DNA sequence of the marker gene directly. Total DNA from the genetically modified plant is digested with restriction enzymes each of which cuts the DNA in a precisely defined manner. The resulting DNA fragments are separated and reacted with the DNA sequence of the marker gene that has been tagged with radioactive or, increasingly commonly, a chemical label. Only the sequence of the marker gene will react with the labelled probe to give a complex that can easily be detected and quantified.

Where the DNA sequence of the marker gene is known from previous work, small fragments of it (oligonucleotide primers) can be synthesised. Under appropriate PCR conditions these primers when incubated with DNA from the genetically modified plant result in repeated and selective amplification of the marker gene with increases of several-million fold possible.

Concerns have been expressed about the use and safety of selectable markers in GMOs released into the environment and also incorporated in foodstuffs. For example, over 30 species of plant have been modified with genes encoding resistance for 9 different antibiotics used in human and veterinary medicine.

CONTAINMENT AND COST

Comprehensive regulations and procedures to deal with laboratory activities and the containment of GMOs in UK laboratories will shortly come into force (Ratledge, 1993). To a certain extent, the Regulations redefine terms. Examples of the techniques which constitute genetic modification are as follows:

(i) recombinant DNA technique consisting of the formation of new combinations of genetic material by the insertion of nucleic acid molecules, produced by whatever means outside the cell, into any virus, bacterial plasmid (autonomously replicating DNA circle) or other vector system so as to allow their incorporation into a host organism in which they do not occur naturally but in which they are capable of continued propagation;

(ii) techniques involving the direct introduction into an organism of heritable material prepared outside the organism, including micro-injection, macro-injection and micro-encapsulation;

(iii) cell fusion (including protoplast fusion) or hybridization techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

For the sake of common sense and expedience, conjugation, transduction, transformation or any other natural process, polyploidy induction and <u>in vitro</u> fertilization do not constitute genetic modification if they do not involve the use of recombinant DNA molecules or GMOs. Likewise, the Regulations shall not apply to the following techniques of genetic modification if they do not involve the use of GMOs as recipients or parental organisms:

(i) mutagenesis;

(ii) the construction and use of somatic hybridoma cells (i.e. for the production of monoclonal antibodies);

(iii) cell fusion (including protoplast fusion) of plant cells where the resulting organisms can also be produced by traditional breeding methods;

(iv) self-cloning of non-pathogenic, naturally occurring micro-organisms with proven histories of safe use and no known adverse consequences in the environment - the so-called Group I micro-organisms where it is necessary that the vectors and inserts should also be well-characterized and free from harmful sequences and should in themselves be poorly mobilizable;

(v) self-cloning and non-pathogenic, naturally occurring organisms other than micro-organisms. Such organisms (i.e. plants and animals) must be as safe in the containment facility as any recipient or parental organism.

Three levels of containment are envisaged for Group II organisms (those that do not conform to the definition of Group I microorganisms) and certain procedures involving Group I, depending upon the evaluation of the risk assessment.

Individual scientists are legally obliged to carry out scale-dependent risk assessments of their work which will then be scrutinised and approved by a local safety committee. The Health and Safety Executive may be involved and will charge a fee for their services and consent. Attention will also have to be paid to transport, storage and destruction of GMOs.

Historically, the UK has a superb safety record of containing and monitoring GMOs. The new Regulations may convince the public that the most stringent conditions are applied. Rather like other legal and statutory aspects of health, safety and the environment, though, there is an exquisite balance to be struck between unfettered irresponsibility and high-cost constraint, demotivation and export of intellectual property elsewhere. Resource-strapped organisations will definitely be ineligible for involvement with GMOs unless they become associated with regional centres specifically designed to meet ever-changing standards imposed by the UK or European Communities.

RELEASE OF GMOs AND THEIR PRODUCTS TO THE ENVIRONMENT AND FOR FOODSTUFFS

By and large there is relatively little opposition to using genetic engineering in most areas of health care, but as the technology is finding application in food production, storage and processing there is widely expressed consumer concern about safety. Some GMOs and their products will continue to be contained strictly within the laboratory environment, others will be monitored closely over a long period to examine their suitability for release into the environment and for consumption. A case-by-case analysis will be carried out in every instance.

Safety is not absolute; accidents, exceptional circumstances incidents and testing occur. Anything is possible. Without resorting to examples of road transport policy, holidays in Florida, and the consequences of oxidative processes in living organisms, the nub of the argument is one of setting the level of acceptability of risk. In this instance we are considering the risk of all products arising from genetic engineering, and the risk is typically classified according to effects on mankind.

By way of definition, hazard is a situation that may lead to harm or loss; risk is the chance in quantitative terms of a defined hazard occurring.

Biologists appreciate that there is natural transfer of genes controlling the formation of toxic principles in plants as well as desirable features.

For the most part, species are not static genetically. By natural selection, for example, resistances to a range of adverse xenobiotics can be developed.

One huge problem is that of forecasting performance "in the field". We are not knowledgeable about selection pressures operating on organisms, nor about unique recombination events likely to occur. Frankly, it is not feasible for any committee or individual to assess risk for all the possible combinations of genes. Assessment can only come from experimentation and monitoring, employing a battery of scientific disciplines. To date, release experiments have not been problematical. Even naturally occurring mobile DNA elements are limited in their natural hosts, and can be regarded as excessively promiscuous. In his recent article, Wilson (1993) cites that an analysis of 393 defined field trials of transgenic plants (25 species) between 1986-1991 (in 21 countries) reveals that 50 involved "virus-resistance" traits. Field releases have shown that coat-protein-mediated protection may not behave as predicted in laboratory and growth-chamber experiments; generally, there is greater susceptibility to virus challenge.

Selectable marker genes, especially relating to the potential impact of antibiotic resistance, raise questions about safety. The potential for uncontrolled gene transfer in the intestual tract, soil or by cross-fertilization, or for example herbicide resistance leading to the creation of weeds have received the most attention. What little information is available would not indicate unacceptable risk. Obviously, a great deal of research is still required to quantify risk, if any, and to make recommendations on the use of marker genes. With time, their use will in any case decline, essential markers may need to be inactivated or eliminated prior to release or consumption of the transgenic organism.

Organisational structures to monitor GMO release are already in place in many countries. Much of the real work, though, is labour-intensive, from removal of flowers to stop breeding of transgenics with other plants (especially weeds), surveying experimental sites to eliminate propagules (seeds tubes etc.) in the seasons following the experiment, and monitoring gene flow through ecosystems. It is always a good policy for any country to monitor its vegetation anyway. I am deeply suspicious about requirements for "analysis of benefit" prior to permission being given for the release and use of GMOs. Just who sets the criteria of benefit and performs the analysis? Central planning can be debilitating when prudency and responsible care are the objectives.

Genetically Engineered Foods

In the UK, the assessment of the safety of foods which are themselves GMOs or which are produced in processes involving GMOs, is a part of the remit of the Advisory Committee on Novel Foods and Processes. A decision-tree scheme has been devised to pose a series of questions which indicate the type of information required for individual submissions to the Committee. Food or components of food derived from GMOs must be as safe as, or safer than, their traditional counterparts. The Committee is refreshingly open, as are nearly all scientific committees, with regard to the advice it gives to Government and the reasoning behind that advice. Related committees include the Food Advisory Committee, the Committee on Toxicity and the Advisory Committees on Genetic Modifications and Releases to the Environment. Their deliberations range from food labelling to the nature of research. They function well and reinforce the confidence of the consumer that standards and proper controls are in place in a democratic environment. I very much welcome the very recent

sensible report of the Ethical Committee on Genetic Modification of Food, chaired by the Reverend Dr John Polkinhorne (1993).

Public Attitudes

Voters comprise heterogeneous groups who determine the political, industrial and economic climate of democratic countries. Their taxes and those of private companies support R&D programmes in the public sector. They are also consumers who should be free to exercise choice. Scientists should be providing them with factual basis for reaching informed decisions.

In contrast to healthcare, applications of modern biotechnology to food and the environment are greatly influenced by the level of education, perceived social and ethical issues, as well as reaction, frequent irrational, responses towards non-medical sciences.

There are also objections at a secondary level to the role of multinational companies carrying out genetic engineering and failing to take adequately into account the impact of their activities on the less-developed world or playing one economy off against another. Although not necessarily associated with religious organisations, there is also the oft-cited "unnatural" or "ungodliness" aspect of science replacing natural functions, generating chimaeric organisations, or fiddling with life for profit.

Ignorance of science and technology, as much as ignorance of business, leads to fear, anxiety and reluctance to fund research and development projects. Pressure groups of all kinds are formed. It seems that the public derive most of their limited understanding of science through the arts-dominated media, especially television, where all too often artistic license embellishes scientific observation with imaginative doom-laden claptrap. This must cause the scientific and advanced industrial communities a measure of introspection. Healthy scientific scepticism, questioning, sharp debate, experimentation and wide-ranging open-minded interpretations and conclusions are the stuff of science. So is presentation. There can be no room for indolence nor ineptitude. Scientists, like the public, cover a spectrum of views and attitudes and are difficult to organise except into cliques. The public must realise that it is entirely technology-dependent. Scientists in turn accept justifiable control as much as the financial backing. I am worried that the foucault pendulum is swinging towards harsher controls which cannot be sustained in the longer term even though it is unfashionable to argue against any moves restraining science. Sometimes, pressure groups have vested political interests or social engineering at heart. Nonetheless, a balance-point must be reached, taking into account illogical fears, damage to the environment, healthcare, and the need for science. The need for genetic engineering is irrefutable. It is how we do it that we must get right.

At this juncture, there is a view that only by being aware of obvious benefits to the consumer (e.g. increased safety because of reduced natural toxins, lower costs, post- and disease-free produce, better and more consistent quality etc.) or to the environment (e.g. reduced pesticide inputs, bioremediation etc.) will there be general acceptance to genetic engineering. Fewer problems are experienced with plant-plant than with plant-microbe transgenetics; plant-animal and animal-animal combinations, most notably where "human" genes are concerned can provoke virulent public and pressure group reactions. The greatest level of acceptance will be for transgenic plants used for non-food purposes. Meanwhile, there will be a plethora of legislative barriers.

CURRENT APPLICATIONS OF BIOENGINEERING

Aided by the rapid uptake of biotechnology in the higher education sector, and relatively crude but accurate assessments of its potential by decision-markers in government and private industry, genetic engineering of crop plants is a world-wide phenomenon. Selective herbicide resistance to aid better crop management; introduction of plant-derived insecticidal genes (e.g. protease inhibitors); introduction of characteristics associated with resistance to pests, diseases, abiotic and biotic stresses; enhanced quality (e.g. amino acid composition); production of engineered oils, proteins, carbohydrates, enzymes etc. are examples of projects currently underway using several crops or related species.

The diagnosis and quantification of disease organisms are increasingly reliant on biotechnology, as are studies on the relationships between different races, pathotypes and virulence groups. To investigate the mode of action and effectiveness of control agents requires the new technologies.

Plant breeding is one of the leading beneficiaries of genetic engineering. All parts of normal breeding schedules are being revolutionised, form describing the genetic architecture of parental material, overcoming natural breeding barriers and selections, to propagation, prediction of performance and identifying more accurately the added-value properties of the progeny. Speed is of the essence, so is protection of intellectual property.

New plant varieties arising from traditional breeding methods are protected in many countries by plant variety (or breeder's) rights (PVR), without recourse to patent law. Patents are now being granted for the protection of recombinant methods for the production of transgenic plants and their resultant products. Attempts are being made to harmonise patent law and practice internationally. Ethical concerns are expressed about patenting life-forms and claiming ownership. There is freedom to research under both patent and PVR law, but freedom to commercialise is complex, and therefore plant breeders using modern technologies seek protection of both types for law.

Patent protection is unlikely to affect access to existing germplasm and traditional varieties. Genetic resources and diversity are internationally seen as the common heritage of humankind. Biotechnology adds to genetic diversity. Counterarguments centre on the farmer's privilege to save seed to produce subsequent crops without royalty payments to recoup R&D costs, abuse of monopoly provisions, "ordre public" and the public interest, and also the nature of more discovery. Unfortunately for all concerned, a patent of invention does not guarantee a reward for the inventor; simply put, it gives an opportunity for the inventor or patent proprietor to profit from the invention where there is a profitable market for it. Secrecy in some cases is the best commercial protectant in the short term.

For transgenic plants to achieve a small portion of their potential, basic studies will need to expand on the factors (e.g. promoters etc.) regulating the expression of introduced genes in different organs and tissues at various phases of growth and differentiation. Industry would be assisted by studies on the biosynthesis and degradation of complex natural polymers such as lignin, cutin, suboin and cellulose, manifestations of cell differentiation, and the cellular components of industrial relevance. Single gene studies will give way to polygenic linked constructions.

Bioremediation could have a highly publicity profile for genetic engineering to assist in measures to reverse environmental degradation. Most current research is initially concerned with the construction of microorganisms that can degrade, on command, oil and organic xenobiotics such as polychlorinated biphenyls. Future research is likely to include the production of transgenic plants that can withstand abiotic "stresses" such as high salinity, heavy metal and radionuclide contamination, sewage and factory effluents.

Dinomania apart, the retrieval of nucleic acids from fossils and preserved tissues is of merit for evolutionary studies of all kinds, and for research on adaptation to climates, predation and disease.

Protection of Crop Plants - The Virus Example

Crop protection deserves conferences of its own. A daunting matrix of pests and diseases, vector systems, host types, mechanisms of infection and infestation, control measures, economics, impact assessments, and variability confronts every reviewer. Biotechnology finds its greatest sophistication in crop protection in the area of virology, for understandable reasons connected to viral modus operandi.

Most plant species are naturally resistant to the majority of the 675 or more plant viruses currently identified. All crops, however, are prone to significant yield and quality losses caused by one or more viruses. Plant viral genomes are plastic and resistance-breaking virus strains are rapidly produced in monocultural agricultural systems with intense selection pressures. This, in turn, causes difficulties for plant breeders attempting to introduce dominant and durable resistance genes: there may be true immunity, subliminal infection or symptomless tolerance to infection. No resistance genes per se have been characterised to date. For many years, there have been observations that infection with mild, symptomless or attenuated strains of viruses could "cross-protect" a range of field crops against closely related, but severely pathogenic virus strains. Virus-resistant crops have been created in many countries by genetically engineering them to express part of a viral genomic or virus-associated sequence. Cognate projects on virus control measures are aimed at the virus vectors (nematodes, insects and fungi) and the molecular features determining virus transmission and replication.

Transgenic plants expressing viral-derived sequences have been discussed as sites for hyper-evolution of pathogenic viruses through recombination events. There is no supporting evidence for this. Any long-term genetic or epidemiological effects would seem remote.

An exciting concept for future work is the protection against fungal, bacterial and viral diseases by expressing appropriate antibodies in transgenic plants. Eventually, we would like to be in a position to solve resistance gene construction and action.

CONCLUSION

Genetic engineering is here to stay. No doubt the introduction of the wheel was foreseen by some to require road traffic legislation as a way to delay development. The technology is clever and takes science and industry

into a new phase of opportunity. Our shared responsibility is to get it right.

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Session 2

New Herbicides and Plant Growth Regulators

Chairman	Dr A D DODGE
Session Organiser	Dr L G COPPING
Papers	2-1 to 2-10

F8426 - A NEW, RAPIDLY ACTING, LOW RATE HERBICIDE FOR THE POST-EMERGENCE SELECTIVE CONTROL OF BROAD-LEAVED WEEDS IN CEREALS

W. A. VAN SAUN, J. T. BAHR, L. J. BOURDOUXHE, F. J. GARGANTIEL, F. W. HOTZMAN, S. W. SHIRES, N. A. SLADEN, S. F. TUTT, K. R. WILSON

FMC Corporation, Agricultural Chemical Group,
P.O. Box 8, Princeton, New Jersey, U.S.A. 08543
and
FMC Corporation N.V.
Avenue Louise, 480 B9, 1050 Brussels, BELGIUM

ABSTRACT

F8426, is a new selective cereal herbicide discovered and under development at FMC Corporation. It is an inhibitor of protoporphyrinogen oxidase. Applied post-emergence, F8426 results in rapid desiccation of sensitive weed species. Translocation is limited. Field testing over several years in the United States, the United Kingdom, France, Germany, the Philippines, Australia, and selected other countries, indicates that F8426 will control a wide range of broad-leaved weeds with good tolerance to wheat, barley, and rice. In Europe, F8426 is especially effective against *Galium aparine, Lamium purpureum,* and *Veronica* spp. In the U.S., it is effective against most major broad-leaved weeds in wheat, including *Kochia scoperia*, *Salsola kali*, *Chenopodium album*, *Amaranthus retroflexus*, and a wide range of winter annual mustards.

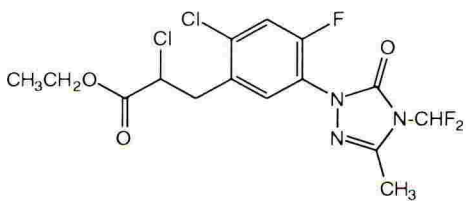
INTRODUCTION

Although the cereal grower is generally well served by a variety of pre- and post-emergence herbicides which are applied either individually or in combination for broad spectrum weed control, a few species such as *Galium aparine*. and *Veronica* spp., which are difficult to control, continue to be a problem. In addition, the increasing incidence of weed populations resistant to ALS inhibitors requires alternative weed control products.

F8426, a new post-emergence herbicide discovered (Poss, 1992) and in development at FMC Corporation, controls a diversity of broad-leaved weeds at field application rates between 4 and 35 g/ha, and is particularly effective on *Galium aparine*, *Abutilon theophrasti*, *Ipomoea hederacea* var. *hederacea*, *Chenopodium album*, and on several important mustard species.

PHYSICAL AND CHEMICAL PROPERTIES

Structure:



Chemical name: (IUPAC)	Ethyl 2-chloro-3-[2-chloro-4-fluoro-5-(4-difluoromethyl-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl)phenyl]propionate
Chemical formula:	$C_{15}H_{14}Cl_2F_3N_3O_3$

Molecular weight:	412.2
Physical description:	Viscous yellow liquid
Density:	1.457 g/cm^3 at 20°C
Melting point:	-22.1°C
Boiling Point:	350-355°C @ 760 mm/Hg
Flash Point:	over 110°C
Vapor pressure:	1.2 x 10^{-7} mm Hg @ 25°C 5.4 x 10^{-8} mm Hg @ 20°C
Solubility: (in water)	12 µg/ml at 20°C 22 µg/ml at 25°C 23 µg/ml at 30°C
Octanol/Water Partition Coefficient:	log K_{OW}= 3.36

TOXICOLOGY

Technical F8426:

Standard Ames mutagenicity:	Negative
Acute oral LD_{50}(rat):	5143 mg/kg (female)
Acute dermal LD_{50}(rat):	>4000 mg/kg
Skin irritation (rabbit):	Non-irritating
Eye irritation (rabbit):	Minimally irritating
Skin sensitisation (guinea pig):	Non-sensitising

FATE IN SOIL AND THE ENVIRONMENT

Mobility

F8426 is strongly absorbed to sterile soils (K_{OC}= 750±60 at 25°C). In non-sterile soils F8426 is rapidly converted to the free acid. The free acid has low soil binding (Koc=15-35 at 25°C and pH 5.5).

Degradation

Based on laboratory tests, loss of F8426 in soil appears to be primarily by microbial degradation. F8426 is not susceptible to photodecomposition nor volatility following application to soil.

Persistence

F8426 appears to be non-persistent in the soil. In the laboratory the soil half-life of F8426 is a few hours. It degrades to the free acid, which in turn has a half-life of 2.5 to 4.0 days in a biologically active loam soil. A value of 10 days was obtained from a less biologically active loam soil. The field half-life of F8426 relative to standard herbicides has not yet been determined.

MODE OF ACTION

Mechanism of Action

F8426 controls weeds through the process of membrane disruption. Laboratory experiments indicate F8426 acts by the same mechanism as the diphenyl ethers and as F6285 in which membrane disruption is initiated by the inhibition of protoporphyrinogen oxidase in the chlorophyll biosynthetic pathway leading to a subsequent build-up of phytotoxic intermediates (Matringe et al., 1989; Witkowski & Halling, 1989). Plants treated with F8426 become necrotic and die shortly after treatment. Initial symptoms are observed within hours and death within a few days.

Uptake / Movement

F8426 is rapidly taken up by foliage of treated plants, with rainfastness achieved within 15 minutes of application. Symplastic phloem movement is assumed to be limited, based on rapid foliar desiccation, although some species are well-controlled even without total spray coverage.

FORMULATIONS

Type:	Water-dispersible granule
Active ingredient:	50% wt/wt
Properties:	
Flash point:	>93°C
Specific gravity:	g/ml
Type:	Emulsifiable concentrate
Active ingredient:	240 g/ l 22.5% wt/wt
Properties:	
Flash point:	>93°C
Specific gravity:	1.067 g/ml

EFFICACY

Field

F8426 was tested in small plot field trials in the United States, Canada, Europe, selected east Asian countries and Australia during several seasons. The results presented here summarise the United States and European programs during the three testing seasons.

Field testing conducted in the United States indicates that F8426 controls (≥85%) a diversity of broad-leaved weeds common in cereals when applied post-emergence at a rate of 35 g/ha. For some species F8426 does not kill the growing point of all plants present.

Dicotyledonous species controlled at 35 g AI/ha F8426 (U.S.)

Abutilon theophrasti	*Amaranthus retroflexus*	*Amsinckia intermedia*
Camelina microcarpa	*Chenopodium album*	*Chorispora tenella*
Descurainia pinnata	*Descurainia sophia*	*Erodium cicutarium*
Erysimum repandum	*Kochia scoparia*	*Salsola kali*
Sinapis arvensis	*Solanum nigrum*	*Thlaspi arvense*

Polygonum convolvulus was the most important weed species not adequately controlled by F8426 in the U.S. in cereals.

In Europe, F8426 provides control or strong suppression of several important weeds prevalent in cereals when applied postemergence. At an application rate of 20 g AI/ha, F8426 controlled *Galium aparine, Veronica hederfolia, Veronica persica, Capsella bursa-pastoris, Chenopodium album,* and *Lamium purpureum.*

Dicotyledonous species controlled at 20 g AI/ha F8426 in spring cereals (Europe)

Capsella bursa-pastoris	*Galium aparine*	*Veronica hederifolia*
Chenopodium album	*Lamium purpureum*	*Veronica persica*
Galeopsis tetrahit		

In rice in Asia, F8426 is effective on annual sedges, annual broad-leaves, and *Echinochloa* spp.

CROP TOLERANCE AND ROTATIONAL SAFETY

Wheat is the major crop demonstrating good tolerance toward F8426 herbicide. Barley, rice, and other cereals have also shown good tolerance (up to 40 g/ha). Good crop tolerance has been observed in corn and soybeans at rates below 35 g/ha. As pre-emergence weed control requires rates from 120-500 g/ha, and soil persistence is short, F8426 should present excellent crop rotational safety.

Tolerant		Marginally tolerant	Not tolerant	
Wheat	Rye	Corn (*Zea mays*)	Cotton	Sunflower
Barley	Rice	Soybean	Potato	Sugar Beet
Oats		Sorghum		

CONCLUSIONS

In summary, F8426 is a low rate, non-residual, contact herbicide that effectively controls many important weeds in cereal culture. It is also effective against sulfonylurea-resistant populations of important weeds such as *Kochia scoparia.* This new herbicide will be a valuable addition to our arsenal of cereal herbicides to aid growers in the safe and effective control of problem weeds.

ACKNOWLEDGMENTS

We gratefully acknowledge the contributions of the FMC biologists who conducted the field trials, and all of our colleagues at FMC who contributed to the discovery and progress toward development and commercialization of F8426.

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KIH-9201, A NEW LOW-RATE POST-EMERGENCE HERBICIDE FOR MAIZE *(ZEA MAYS)* AND SOYBEANS *(GLYCINE MAX)*

T. MIYAZAWA, K. KAWANO, S. SHIGEMATSU

Kumiai Chemical Industry Co., Ltd., Life Science Research Institute, Kikugawa, Shizuoka, Japan.

M. YAMAGUCHI and K. MATSUNARI

K-I Chemical Research Institute Co., Ltd., Fukude, Iwata, Shizuoka, Japan.

P. PORPIGLIA

CIBA-GEIGY Corporation, Greensboro, NC, USA.

K. G. GUTBROD

CIBA-GEIGY Ltd., Basle, Switzerland

ABSTRACT

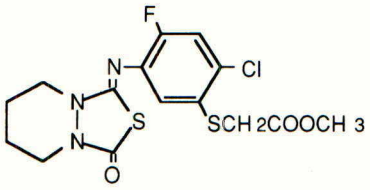
KIH-9201, methyl[2-chloro-4-fluoro-5-[(tetrahydro-3-oxo-IH, 3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene) amino]phenol] thio] acetate is a new, selective, post-emergence herbicide for control of certain annual broadleaf weeds in maize (*Zea mays*) and soybeans (*Glycine max*). Outstanding control of *Abutilon theophrasti* in the 1 to 8 leaf growth stages consistently occurs under field conditions at 5 g AI/ha. KIH-9201 also provides good to excellent control of *Amaranthus retroflexus*, *Chenopodium album*, and other species at 5-10 g AI/ha. Maize and soybeans exhibit good tolerance to over-the-top applications of KIH-9201. Field and laboratory results to date indicate the favorable environmental and toxicological properties of this new herbicide.

INTRODUCTION

Selective broad spectrum control of broadleaf weeds in maize and soybeans is needed for maximum yield potential, but most common herbicides do not control the complete weed spectrum. KIH-9201 is a potent new herbicide for maize and soybeans discovered by Kumiai Chemical Industry Co., Ltd.. It exhibits high activity on problem broadleaf weeds with good crop safety. KIH-9201 is currently being jointly developed in the U.S. by Kumiai and CIBA-GEIGY Corporation under the code number, CGA-248 757. This paper describes the chemical, physical and biological properties of KIH-9201.

CHEMICAL AND PHYSICAL PROPERTIES

Chemical formula:



Chemical name (IUPAC)	: methyl [[2-Chloro-4-fluoro-5 [(tetrahydro- 3-oxo-IH, 3H- [1,3,4] thiadiazolo [3,4-a] pyridazin-1- ylidene) amino]-phenyl] thio] acetate
Common name	: not yet given
Code number	: KIH-9201, CGA-248 757
Empirical formula	: C_{15} H_{15} $ClFN_3$ O_3 S_2
Molecular weight	: 403.88
Appearance	: white powder
Melting Point	: 104.6° C
Volatility	: 9.2 x 10^{-6} Pa (very low)
Solubility	: 0.64 mg/liter in water at 29°C

TOXICOLOGY OF TECHNICAL MATERIAL

Acute toxicity	Oral LD50 rat		>5000 mg/kg
	Dermal LD50 rat		>2000 mg/kg
Irritation	Skin, rabbit		non-irritant
	Eye, rabbit		slight irritant
Mutagenicity	Non-mutagenic in Ames Test		
Teratogenicity	Non-teratogenic (rat and rabbit)		
Subchronic toxicity	No effect level,	rat-	10 ppm (male)
			100 ppm (female)
		mouse-	10 ppm

SOIL BEHAVIOR

Partition coefficient n-octanol/water	log K 3.55
Soil degradation (parent, 2 soils)	t1/2 at 29°C <7 days (50% field capacity)

MODE OF ACTION

KIH-9201 acts rapidly on foliage of sensitive species by inducing an accumulation of protoporphyrins, which enhances peroxidation of membrane lipids. This leads to irreversible damage of membrane structure and cellular function in sensitive weeds. Light and oxygen are required for herbicidal activity. Symptoms include leaf necrosis which is often apparent within 24-48 hours.

MATERIALS AND METHODS

Greenhouse trials with KIH-9201 were conducted in Japan using a 120 g AI/l EC formulation. Post-emergence applications were made in 250 l/ha spray volume with a non ionic surfactant at 0.25% V/V. Test plants were in the 1-5 leaf stage at application. Visual evaluation of herbicidal effects were made 20-30 days after treatment as per cent activity. Pre-emergence soil applications were also evaluated.

Field experiments have been conducted since 1988 in the United States and Japan, as well as in other countries. Maize and soybean trials contained 2 to 4 replicates, with a plot size of 10-30 m^2. Standard fertility and maintenance programs were used. KIH-9201 was applied in a spray volume of 200-400 l/ha with a non ionic surfactant. Pre-emergence trials were also conducted. Standard commercial herbicides were included at their recommended rates and additives for comparison. Herbicidal activity and crop phytotoxicity were assessed visually at various intervals after treatment using a 0-100 per cent scale on crops and weeds.

RESULTS AND DISCUSSION

Greenhouse Trials

Excellent control of many weed species was obtained from rates of 2.5 to 10 g AI/ha. Table 1 shows the activity on nine weeds in greenhouse trials. Soybeans in the first to third trifoliate leaf stage and maize in the 2-5 leaf stage exhibited excellent tolerance to KIH-9201 at these rates.

TABLE 1. KIH-9201 post-emergence herbicidal activity and crop tolerance in greenhouse trials.

	% Activity		
Plant Species	10	5	2.5 (g AI/ha)
Abutilon theophrasti	100	100	100
Amaranthus retroflexus	100	100	100
Chenopodium album	100	100	90
Ipomoea hederacea	96	85	63
Ipomoea purpurea	90	68	41
Polygonum lapathifolium	65	35	16
Portulaca oleracea	78	53	22
Sesbania exaltata	93	90	78
Xanthium strumarium	100	100	92
Maize	4	0	0
Soybeans	2	0	0

Other greenhouse results show *Amaranthus viridis*, *Datura stramonium*, *Solanum nigrum*, *Sida spinosa* and *Commelina communis* to be susceptible to 10 g AI/ha. Species tolerant to this rate include *Cassia tora*, *Bidens pilosa*, *Stellaria media*, *Setaria viridis*, *Digitaria ciliaris*, *Echinochloa crus-galli* and *Sorghum halepense*. At higher application rates, crop foliage present at application was burned, but new growth was not affected.

Field Trials in USA

KIH-9201 applied post-emergence in maize and soybeans at 5 to 10 g AI/ha showed selective control of problem broadleaf weeds, such as *Abutilon theophrasti*, *Amaranthus retroflexus* and *Chenopodium album* (Table 2).

Table 2. Average per cent activity of KIH-9201 at 5 or 10 g AI/ha on maize, soybeans and broadleaf weeds (1 evaluation/trial taken 14-35 DAA).

	% Activity (No. of trials)	
Plant Species	5 g AI/ha	10 g AI/ha
Maize	1.4 (31)	3.6 (20)
Soybeans	6.1 (71)	8.6 (69)
Abutilon theophrasti	95 (38)	99 (31)
Chenopodium album	79 (40)	88 (39)
Amaranthus retroflexus	66 (30)	79 (21)

KIH-9201 exhibits superior post-emergence application timing flexibility on *Abutilon* and *Chenopodium*, when applied at 5 to 10 g AI/ha, as compared to several standards (Table 3). At the lowest rate tested, KIH-9201 gave complete control of *Abutilon*, regardless of growth stage. On *Chenopodium*, KIH-9201 performed better than most standards. Maize and soybean selectivity was not affected by application timing.

Table 3. Influence of stage of weed growth on performance of KIH-9201 compared to commercial standards (3-12 weeks after application).

			Average % Control (No. of trials)			
Product	Rate		*Abutilon* (5)		*Chenopodium* (3)	
	(g AI/ha)	Stages(cm)	2-15	10-51	1-5	2-15
KIH-9201	5		100	100	85	89
KIH-9201	10		100	100	97	97
Acifluorfen	560		54	66	79	79
Chlorimuron	13		86	82	37	69
Imazethapyr	70		86	88	63	58
Bentazone	1120		92	87	87	90
Thifensulfuron	4.4		90	91	90	96

Pre-emergence applications resulted in almost no effect on all crop and weed species tested, even from application rates up to 120 g AI/ha. Based on this and limited recropping data, no limitations in rotational flexibility are expected.

The rapid activity of KIH-9201 enhances the early performance when mixed with certain combination products. Table 4 shows that this effect is still manifest at 21-42 days after application where tank-mixes of KIH-9201 with commercial standards improves activity on the key weed species controlled by KIH-9201 alone (e.g. *Abutilon* and *Chenopodium*) without negatively affecting activity on weed species well controlled by the standards (eg *Xanthium*).

Table 4. Efficacy of KIH-9201 at 5g AI/ha alone and in tank-mixtures (t-mix*) with five commercial standards in soybeans (2-4 trials per species; evaluations 21-42 DAA)

Product	Rate (g AI/ha)	Average % Control Across Trials					
		Abutilon (2)		*Chenopodium* (3)		*Xanthium* (4)	
		alone	t-mix*	alone	t-mix*	alone	t-mix*
KIH-9201	5	100	-	84	-	38	-
Acifluorfen	840	94	100	84	93	68	65
Bentazone	1120	99	100	71	80	82	92
Chlorimuron	13	82	100	26	67	87	86
Imazethapyr	70	85	99	50	87	86	93
Thifensulfuron	4	79	100	74	94	65	65

These results show the utility of KIH-9201 to broaden the spectrum of various standards to ensure control of problem species such as *Abutilon*, *Chenopodium* and possibly others. Further work is underway to confirm the enhanced control of these and other species in maize and soybeans with the appropriate standards.

CONCLUSIONS

1. Greenhouse results obtained with KIH-9201 demonstrated post-emergence maize and soybean selectivity and herbicidal activity on certain problem broadleaf weeds when applied in the 5 to 10 g AI/ha range.

2. Field results confirmed the post-emergence maize and soybean selectivity of KIH-9201 at 5-10 g AI/ha and efficacy on important broadleaf species such as *Abutilon theophrasti*, *Chenopodium album* and *Amaranthus retroflexus*.

3. The high level of activity allows post-emergence applications with appropriate additives over a wide range of growth stages of *Abutilon* and *Chenopodium*, while maintaining crop selectivity.

4. KIH-9201 can be used in combination with acifluorfen, bentazone, chlorimuron, imazethapyr and thifensulfuron to ensure control of such problem species as *Abutilon*, *Chenopodium* and others for broad spectrum control.

5. Initial trials show no problems with rotational flexibility because of little pre-emergence activity, even from excessive rates.

6. The very low application rate associated with rapid soil degradation and low soil activity indicate that KIH-9201 has favorable environmental properties.

7. Trials underway indicate KIH-9201 to have a favorable toxicological profile.

ACKNOWLEDGEMENTS

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THIDIAZIMIN - A NOVEL HERBICIDE FOR USE IN CEREALS

R. WEILER, G. JOHANN, M. GANZER, M. MACH

Schering AG, Agrochemical Division, 13342 Berlin, Germany

ABSTRACT

Thidiazimin is a novel herbicide discovered by Schering AG. The primary use is for post-emergence control of broad-leaved weeds in winter cereals. All toxicological studies completed to date are favourable. Physico-chemical parameters and initial environmental fate studies indicate an environmentally favourable behaviour of the compound. Field trials conducted since 1989 have demonstrated good post-emergence activity at rates between 20 and 40 g AI/ha against a wide range of important broad-leaved weed species. The compound is particularly effective against *Lamium purpureum, Veronica hederifolia, Veronica persica,* and *Viola arvensis*. Thidiazimin has demonstrated good safety to all tested cereal crops.

INTRODUCTION

Thidiazimin was discovered in 1987 by Schering AG. In early glasshouse and field experiments the compound applied at low dose rates in post-emergence provided excellent control of important broad-leaved weed species combined with particularly good selectivity in cereals. Since 1989 thidiazimin has been evaluated as a post-emergence herbicide against broad-leaved weeds primarily in winter cereals in an extensive field trial programme throughout Europe. This paper describes the main physical and chemical properties as well as the toxicological and environmental profile of thidiazimin and summarizes results from the above mentioned field trial programme.

CHEMICAL AND PHYSICAL PROPERTIES

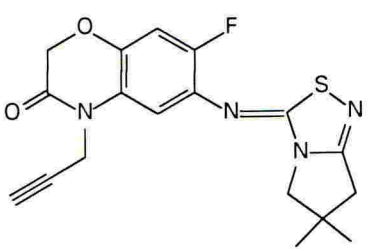
Common name: (ISO proposed) thidiazimin

Code number: SN 124 085

Chemical name (IUPAC):

6-[(3Z)-6,7-dihydro-6,6-dimethyl-3H,5H-pyrrolo[2,1-c][1,2,4]thiadiazol-3-ylideneamino]-7-fluoro-4-(2-propynyl)-2H-1,4-benzoxazin-3(4H)-one

Structural formula:



Molecular formula:	$C_{18}H_{17}FN_4O_2S$
Molecular weight:	372.4
Melting point:	158 °C
Appearance:	colourless crystals
Water solubility:	(6.6 ± 0.5) x 10^{-3} g/l at 25 °C, pH7
Vapour pressure:	5.0 x 10^{-11} Pa at 25 °C
Partition coefficient:	log Pow = 3.0 at pH7
Dissociation constant:	pK_a = 2.6 ± 0.1 (conjugate acid)

TOXICOLOGY (technical material)

Acute oral LD50 (rat):	> 4000 mg/kg
Acute dermal LD50(rat):	» 5000 mg/kg
Inhalational LC50 (rat):	> 4.89 mg/l

Thidiazimin is of very low acute oral and dermal toxicity to rodents. Skin and eye irritancy tests in the rabbit and a Buehler skin sensitisation test in the guinea pig were all negative.

The material was well tolerated in repeat dose studies and was negative in rat and rabbit teratology studies. Thidiazimin has been tested in a range of genetic toxicology assays which showed the compound to be non-genotoxic.

Avian acute and subacute studies show the material to be of low toxicity to birds.

ENVIRONMENTAL CHEMISTRY

Adsorption coefficient (K_{OC}):	700 - 1200 ml/g
Soil dissipation (DT_{50}): (laboratory; depending on soil type)	30 - 70 days

Results of all studies to date point to an environmentally favourable behaviour of thidiazimin. Migration of the compound from the site of application to other environmental compartments is not expected to occur to any significant degree.

MODE OF ACTION

Thidiazimin is a representative of the chemical class of imino-1,2,4-thiadiazoles, a special group of protoporphyrinogen-IX-oxidase-inhibiting herbicides. It is a contact type herbicide with practically no residual activity. Thidiazimin has a very rapid action. Effects become visible as desiccation and necrosis of exposed susceptible plant tissue.

These effects are similar to those caused by other known protoporphyrinogen-IX-oxidase inhibitors such as diphenyl-ethers (Matringe *et al.*, 1989).

MATERIALS AND METHODS

Biological results reported in this paper were obtained from field trials carried out in the years 1991 - 1993 in France, Germany, United Kingdom, Belgium, Denmark, and Italy. In these trials formulated thidiazimin was applied in winter wheat, winter barley, winter rye and triticale post emergence at different timings in autumn (15.10. - 30.11.) or spring (10.03. - 05.04.). The compound was tested at rates of 20 - 40 g AI/ha.

For trial work, technical thidiazimin was formulated as aqueous SC formulations containing either 20 or 100 g AI/l. No differences were observed in biological activity between the formulations. The trial design was randomised block with 3 - 4 replications and plot sizes between 5 and 20 m². The spray volume was 200 - 500 l/ha applied with small plot spraying equipment.

Weed species were either sown across the plots or were present as indigenous populations. Percentage weed control and crop tolerance were assessed by visual assessment, 7 - 30 days after treatment (DAT).

FIELD RESULTS

Crop safety

TABLE 1. Decline of Crop Symptoms following Autumn Application of Thidiazimin (% Damage in Trials with Crop Effects)

Crop	Number of Trials	Rate (g AI/ha)	DAT 7	17	27
Winter wheat	6	20	3	0	0
		30	3	0	0
		40	5	0	0
Winter barley	6	20	10	3	0
		30	12	3	0
		40	14	6	0
Winter rye	4	20	3	0	0
		30	5	2	0
		40	7	4	0
Triticale	4	20	4	1	0
		30	7	2	0
		40	11	4	0

The selectivity margin of thidiazimin at all application timings was good for all tested cereal crops.

In a few trials, especially with winter barley, the compound produced necrotic spots on the youngest leaves of the treated crop. These symptoms are outgrown very shortly after application (Table 1). At the tested rates, thidiazimin did not produce any phytotoxic effect to the crop via root-uptake.

Weed control

TABLE 2. Weed Control following Autumn Application of Thidiazimin (%)

Weed species	Number of Trials	Rate g AI/ha 20	30	40
Broad leaved weeds				
Adonis annua	2	94	97	99
Aphanes arvensis	3	99	99	100
Brassica sp.	8	59	73	80
Capsella bursa-pastoris	19	85	90	95
Galium aparine	41	77	83	89
Lamium purpureum	27	97	99	98
Matricaria chamomilla	28	82	89	90
Myosotis arvensis	14	90	92	95
Papaver rhoeas	11	95	97	98
Sinapis arvensis	3	92	96	98
Stellaria media	47	85	92	96
Thlaspi arvense	3	100	100	100
Tripleurospermum maritimum	10	86	92	97
Veronica hederifolia	16	99	100	100
Veronica persica	15	98	100	100
Viola arvensis	36	89	94	94
Grasses				
Alopecurus myosuroides	10	7	9	10
Apera spica-venti	12	10	12	15
Poa annua	5	8	8	10

Thidiazimin applied post-emergence in autumn (Table 2) or spring (Table 3) at rates between 20 - 40 g AI/ha has good activity against a wide range of broad-leaved weed species. The compound acts very rapidly and is relatively independent of temperature. In particular the important species *Lamium purpureum*, *Veronica hederifolia, Veronica persica* and *Viola arvensis* showed a very high sensitivity to the compound, but also other species such as *Capsella bursa-pastoris*, *Galium aparine*,

Matricaria chamomilla, Myosotis arvensis, Papaver rhoeas, and *Stellaria media* were very effectively controlled.

Best results have been achieved with autumn applications when weeds are still in an early stage of development. On larger weeds a mixture of thidiazimin with suitable herbicides such as urea-herbicides, substituted benzonitriles, sulphonylureas and hormone type herbicides, leads to a complete control of all broad-leaved weeds. Thidiazimin has no significant efficacy against grasses.

TABLE 3. Weed Control following Spring Application of Thidiazimin to Autumn Sown Cereals(%)

Weed Species	Number of Trials	Rate g AI/ha 20	30	40
Broad leaved weeds				
Aphanes arvensis	2	92	94	96
Brassica sp.	5	39	45	51
Capsella bursa-pastoris	11	55	65	72
Galium Aparine	42	90	93	95
Lamium purpureum	26	97	98	98
Matricaria chamomilla	29	63	68	70
Myosotis arvensis	20	78	82	84
Papaver rhoeas	13	71	79	83
Senecio vulgaris	2	97	99	100
Stellaria media	44	79	82	85
Thlaspi arvense	2	83	88	91
Tripleurospermum maritimum	9	73	78	83
Veronica hederifolia	12	89	93	95
Veronica persicaria	24	95	98	98
Viola arvensis	31	92	95	95
Grasses				
Alopecurus myosuroides	6	6	8	9
Apera spica-venti	7	8	11	12
Poa annua	3	5	5	7

CONCLUSION

The field trials have demonstrated that post-emergence application of thidiazimin at rates of 20 - 40 g AI/ha in cereals gives very good and rapid control of a wide range of broad-leaved weeds, including the important species *Capsella bursa-pastoris, Galium aparine, Lamium purpureum, Matricaria chamomilla, Myosotis arvensis, Papaver rhoeas, Stellaria media, Veronica hederifolia, Veronica persica, and Viola arvensis.*

All cereal crops thus far tested are tolerant. Temporary necrosis on young leaves occurred only in a few trials. Specific investigations (not reported here) have shown that these symptoms did not influence the subsequent yields.

At the tested rates the compound does not cause any carry-over effects in following crops.

From the chemodynamic parameters given above the conclusion can be drawn that neither contamination of groundwater resources by leaching nor pollution of neighbouring areas through volatilization should be issues of concern.

Results from the toxicological investigations up to now have been favourable.

The outstanding biological properties described above together with favourable toxicological and environmental data give thidiazimin considerable potential as a new contact herbicide for broad-leaved weed control in winter cereals.

Thidiazimin is an ideal combination partner for established or new cereal herbicides with complementary broad-leaved weed or grass activity.

ACKNOWLEDGEMENTS

We gratefully acknowledge the help and advice of our colleagues in Schering Research, Development and Marketing Departments.

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ET-751: A NEW HERBICIDE FOR USE IN CEREALS

Y. MIURA, M. OHNISHI

Nihon Nohyaku Co.Ltd., Chemical Research Center, 5-1-34 Tsukuda, Nishiyodogawa-ku, Osaka 555, Japan

T. MABUCHI

Nihon Nohyaku Co.Ltd., Biological Research Center, 4-31 Honda-cho, Kawachinagano, Osaka 586, Japan

I. YANAI

Nihon Nohyaku Co.Ltd., Development Division, 1-2-5 Nihonbashi, Chuo-ku, Tokyo 103, Japan

ABSTRACT

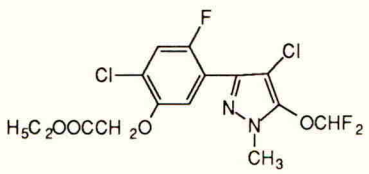
ET-751 is a new selective post-emergence herbicide for use in cereals. The activity spectrum of ET-751 includes *Galium aparine*, *Matricaria inodora*, *Lamium purpureum*, *Stellaria media* and other important broad-leaved weeds. Best results have been obtained in Europe at dose rates between 6-12 g AI/ha to autumn-sown cereals with good crop safety. The compound has potential as a partner to other cereal herbicides which have complementary weed spectra.

INTRODUCTION

ET-751 is a new 3-phenylpyrazole herbicide discovered by Nihon Nohyaku Co.Ltd.. The primary use is for the post-emergence control of broad-leaved weeds in cereals. Field trials conducted in Europe have demonstrated good activity against important weed species under different climatic conditions. This paper describes the chemical, toxicological and biological properties of ET-751, and presents data from laboratory, glasshouse, growth chamber and field tests.

CHEMICAL AND PHYSICAL PROPERTIES

Structure:



Chemical name (IUPAC): ethyl 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetate

Molecular formula : $C_{15}H_{13}Cl_2F_3N_2O_4$

Melting point	:	126.0-127.0°C		
Molecular weight	:	413.18		
Appearance	:	Technical product:	pale brown, crystalline	
Vapour pressure (at 25°C)	:	4.79 mPa		
Solubility (at 25°C)	:	water	:	< 1 ppm
		xylene	:	2.9 %(w/w)
		acetone	:	25.0 %(w/w)
		ethanol	:	1.0 %(w/w)
		ethyl acetate	:	14.5 %(w/w)

TOXICOLOGY (by preliminary studies)

Acute oral (LD50)	rat	:	> 5000 mg/kg
Fish toxicity (LC50,48h)	carp	:	> 10 ppm
	rainbow trout	:	> 10 ppm
Skin irritation	rabbit	:	non irritant
Eye irritation	rabbit	:	slight irritant
Mutagenicity (Ames test)		:	negative

MODE OF ACTION

ET-751 is a new contact herbicide. When applied to foliage, it is readily absorbed into plant tissues, and rapid necrosis or desiccation of stems and leaves is induced in the presence of light. Preliminary studies suggest the accumulation of protoporphyrin IX in plant cells as with diphenyl ether herbicides.

MATERIALS AND METHODS

Glasshouse, growth chamber and field trials

ET-751 was formulated as a 5% EC. The chemical was applied in spray volume of 500 l/ha in glasshouse tests and 300 l/ha in other experiments.

For glasshouse evaluation, seeds of crops and weeds were sown in plastic containers filled with a sandy loam soil. Pre- and post-emergence treatments were coducted on the next day after seeding and when crops were at the GS13 (Zadocks' scale) and weeds were at the 3-5 leaf growth stage, respectively. Visual assessment of herbicidal activity and crop safety were made 21 days after treatment (DAT), using a percentage scale of 0 (no effect) to 100 (completely killed).

Growth chamber experiments were maintained in a 12 h photoperiod at 10-15°C with a light intensity of 450$\mu E/m^2/sec$. Visual assessment was made 56 DAT on each stage of the weed tested.

Field experiments were performed from 1990 till 1991 in Southern England and S.W. France. The field evaluation was carried out as follows, and crop injury was shown as the mean value of those of the cultured varieties:

Application	Country	Crop stage*	Weed stage	Visual assessment Crop DAT	Visual assessment Weed DAT
Early post-emergence	UK	21	cotyledon-4 leaves	184	100
	France	20-21	cotyledon-5 leaves	119	37
Late post-emergence	UK	30-31	6-10	70	42
	France	21-24	4-8	81	46

* Growth stage at the time of treatment (Zadoks' scale).

RESULTS AND DISCUSSION

Glasshouse tests

ET-751 achieved very high activity in the post-emergence treatment against broad-leaved weeds such as *Galium aparine*, *Stellaria media*, *Matricaria inodora*, *Veronica persica* and *Viola arvensis* within a week of treatment, but was relatively inactive against grass weed species such as *Alopecurus myosuroides* and *Avena fatua* and cereal crops (Table 1). Clear selectivity was observed between cereals and broad-leaved weeds. Pre-emergence applications of ET-751 were much less effective with a four fold rate increase required perfect control of susceptible weeds.

TABLE 1. Percentage weed control and crop selectivity with ET-751 applied pre- or post-emergence in glasshouse tests.

Crops and weeds	% Phytotoxicity / Weed control Pre-emergence 6	Pre-emergence 12	Post-emergence 3	Post-emergence 6
	(g AI/ha)			
Wheat (cv. Avalon)	0	0	3	8
Barley (cv. Igri)	0	0	5	12
Alopecurus myosuroides	0	0	8	16
Avena fatua	0	0	5	18
Galium aparine	0	30	100	100
Stellaria media	5	30	100	100
Matricaria inodora	20	50	98	100
Veronica persica	50	80	100	100
Viola arvensis	0	30	80	95

Growth chamber test

The activity of ET-751 was evaluated on various leaf stages of *Galium aparine* at 6-12 g AI/ha. As shown in Fig. 1, *Galium aparine* was controlled with ET-751 at 6 g AI/ha at the 4 leaf stage and at 12 g AI/ha at the 5 to 6 leaf stage.

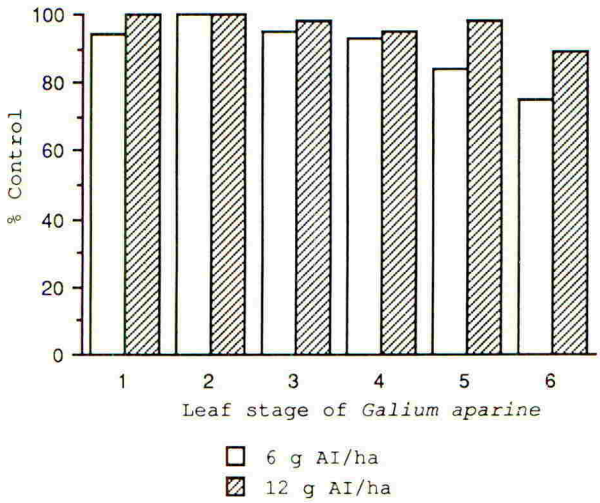


Fig. 1. Percentage control of *Galium aparine* with ET-751 at different leaf stages (growth chamber).

Field trials

In the UK (Table 2), ET-751 applied early and late post-emergence at 12 g AI/ha was effective on important broad-leaved weeds, but ineffective on *Alopecurus myosuroides*. Efficacy on all weed species including *Alopecurus myosuroides* was improved by combining ET-751 with isoproturon. This combination showed superior efficacy to diflufenican + isoproturon (DFF+IPU, Javelin Gold) on *Galium aparine* in early post treatment. Cereal crops were tolerant although transient phytotoxity was observed.

In France (Table 3), ET-751 at 6 g AI/ha gave excellent control of all weeds tested except *Alopecurus myosuroides*. The combination with isoproturon was highly effective to all weeds at 6 + 1540 g AI/ha. This combination was more effective for *Galium aparine* control than DFF + IPU (Quartz GT) in both applications.

Results of early and late post-emergence field trials in the UK and in France demonstrated that ET-751 at 6-12 g AI/ha controlled important broad-leaved weeds. The appropriate rate was dependent on the timing of the treatment. Early post-emergence application gave better results than late application. It is noteworthy that *Galium aparine* control was stable in every treatment. Safety in wheat and barley was excellent at final assessment.

TABLE 2. Percentage weed control and crop selectivity for ET-751 applied early and late post-emergence in the UK.

Crops and weeds	% Phytotoxicity / Weed control ET-751 12		IPU 2500 (2100)#		ET-751+IPU 12+2500 (12+2100)#		DFF+IPU*** 100+2500 (84+2100)#	
			(g AI/ha)					
	T1	T2	T1	T2	T1	T2	T1	T2
Wheat*	0	0	0	0	0	0	0	0
Barley**	0	0	0	0	0	0	0	0
Galium aparine	84	80	0	4	89	88	56	22
Matricaria inodora	89	84	89	100	93	100	96	100
Stellaria media	78	73	85	97	90	91	98	98
Lamium purpureum	99	84	13	82	100	94	76	88
Alopecurus myosuroides	0	0	95	80	99	80	93	80

IPU = Isoproturon
DFF = Diflufenican
T1 : Early post-emergence
T2 : Late post-emergence
* cv. : Mercia, Galahad ,Haven
** cv. : Pipkin, Magie
*** Javelin Gold
# : Rate of T2

TABLE 3. Percentage weed control and crop selectivity for ET-751 applied early and late post-emergence in France.

Crops and weeds	% Phytotoxicity / Weed control ET-751 6		IPU 1540		ET-751+IPU 6+1540		DFF+IPU*** 188+1500	
			(g AI/ha)					
	T1	T2	T1	T2	T1	T2	T1	T2
Wheat*	0	0	0	0	0	0	0	0
Barley**	0	0	0	0	0	0	0	0
Galium aparine	93	93	0	0	97	89	84	27
Matricaria inodora	95	78	94	100	97	100	96	100
Stellaria media	100	95	95	95	100	99	100	100
Lamium purpureum	98	87	80	0	100	88	85	77
Veronica hederifolia	90	91	40	29	89	95	86	86
Alopecurus myosuroides	8	0	89	95	91	92	90	96

IPU = Isoproturon
DFF = Diflufenican
T1 : Early post-emergence
T2 : Late post-emergence
* cv. : Capet, Dore, Centuro
** cv. : Plaisant, Magie
*** Quartz GT

CONCLUSION

ET-751 is a new herbicide for cereals which is highly effective against several important broad-leaved weeds at 6-12 g AI/ha. In particular, it offers excellent control of *Galium aparine* in early post-emergence treatment. ET-751 acts rapidly and the efficacy level is relatively dependent on the growth stage of weeds. It has excellent selectivity to cereal crops although some transient injury has been observed. No carry-over problems are to be expected in succeeding crops due to the lack of pre-emergence activity at 6-12 g AI/ha. ET-751 has potential as a partner to other cereal herbicides which have complementary weed control spectra such as isoproturon.

ACKNOWLEDGEMENTS

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AC 322,140 - A NEW BROAD-SPECTRUM HERBICIDE FOR SELECTIVE WEED CONTROL IN RICE AND CEREALS

M. E. CONDON, T. E. BRADY, D. FEIST, T. MALEFYT, P. MARC, L. S. QUAKENBUSH, S. J. RODAWAY, D. L. SHANER, B. TECLE

American Cyanamid Company, Agricultural Research Division
P. O. Box 400, Princeton, New Jersey 08543-0400 USA

ABSTRACT

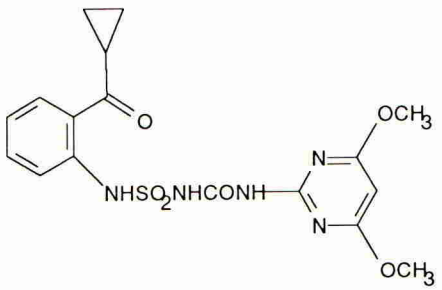
AC 322,140 (1-{[2-(cyclopropylcarbonyl)phenyl]sulfamoyl}-3-(4,6-dimethoxypyrimidin-2-yl)-urea) is a new sulfamoylurea herbicide for weed control in rice and cereal crops. In Japanese paddy rice, 45-60 g AI/ha of AC 322,140 is effective against the major perennial and annual dicotyledonous and sedge weeds. Among the weeds controlled are *Cyperus serotinus, Eleocharis kuroguwai*, and *Sagittaria pygmaea*. In wheat and barley, AC 322,140 controls a number of economically important broadleaf weeds when it is applied pre-emergence, post-emergence in the fall, or post-emergence in the spring at 25-50 g AI/ha. Among the weeds controlled are *Galium aparine, Matricaria spp., Veronica spp., Sinapis arvensis*, and *Brassica napus*. In initial toxicology tests, AC 322,140, an inhibitor of acetohydroxyacid synthase, has been shown to be very safe for mammals and other non-target organisms.

INTRODUCTION

AC 322,140 is a new herbicide (Brady *et al.*, 1991) being developed by the Agricultural Research Division of American Cyanamid for control of a wide range of dicotyledonous and sedge weed species infesting cereals and rice. AC 322,140 is a member of the sulfamoylurea class which is significantly different from sulfonylurea herbicides both in the chemistry of the bridge, and in the nature of the phenyl ring *ortho*-substituent. The unique chemistry of AC 322,140 makes it a highly selective and an environmentally benign herbicide.

CHEMICAL AND PHYSICAL PROPERTIES

Structure:



Common Name: Not yet available

Chemical Name: 1-{[2-(cyclopropylcarbonyl)phenyl]sulfamoyl}-3-(4,6-dimethoxypyrimidin-2-yl)urea

Chemical Formula:	$C_{17}H_{19}N_5O_6S$
Appearance:	Off-white solid
Molecular Weight:	422.0
Melting Point:	170-171° C
Acid Dissociation Constant (pK_a):	5

Water Solubility @ 25°C:

pH	5	6	7	8
ppm	1	3	6	32

K_{ow} @25°C: (Apparent octanol/water partition coefficient)

pH	3	5	6	7	8
K_{ow}	38	111	49	26	5

Stability: {$T_{1/2}$ (days) in 50 mM phosphate buffer}

pH	3	5	6	7	8
$T_{1/2}$	2.2	2.2	5.1	40	91

TOXICOLOGY

Acute oral LD_{50} (mouse):	>500 mg/kg
Acute dermal LD_{50} (rabbit):	>4000 mg/kg
Fish toxicity - 48th LD_{50} (carp):	>10 ppm
Eye irritation (rabbit):	Mildly irritating, with complete recovery within 48 hours
Skin irritation (rabbit):	Non-irritating
Ames mutagenicity:	Non-mutagenic

FORMULATION

Field trials in rice were conducted with extruded clay granules containing 0.05-0.20% active ingredient, and cereal evaluations were conducted with a 65% wettable powder.

MODE OF ACTION

AC 322,140 is a potent inhibitor of acetohydroxyacid synthase (AHAS), the enzyme responsible for the synthesis of the branched-chain amino acids valine, leucine and isoleucine. Inhibition of this enzyme disrupts the plant's ability to manufacture proteins, and this disruption subsequently leads to the cessation of all cell division and eventual death of the plant. Inhibition by AC 322,140 of AHAS isolated from corn cells (Black Mexican Sweet) is compared with three known AHAS inhibitors in TABLE 1.

TABLE 1. AHAS Inhibition (BMS corn cells)

Inhibitor	I_{50}
AC 322,140	0.9 nM
Bensulfuron-methyl	18.9 nM
Chlorimuron-ethyl	6.9 nM
Imazapyr	11.6 µM

EFFICACY

Field trials - rice

AC 322,140 has been extensively field tested in rice by American Cyanamid and its subsidiaries for the past three years, in a total of over 300 field trials throughout the world (Murai, *et al.* 1993; Quakenbush *et al.* 1993). In transplanted rice tests, AC 322,140 at 45-60 g AI/ha applied 2-15 days after transplanting controls many weed species, including the perennial and annual weed species shown in TABLE 2. AC 322,140 showed significant advantages over the standards pyrazosulfuron-ethyl and bensulfuron-methyl on several important weeds such as *Ludwigia prostrata, Lythrum anceps, Monochoria vaginalis, Sagittaria pygmaea, Eleocharis kuroguwai* and *Echinochloa crus-galli*. Grass weeds such as *Echinochloa crus-galli* are suppressed but not consistently controlled. In addition, it also showed activity on an important algae weed, *Spirogyra communis*, on which the standards pyrazosulfuron-ethyl and bensulfuron-methyl have no activity. AC 322,140 has also shown good control of broadleaf and sedge weeds and crop tolerance in water-seeded rice when applied 0-12 days after seeding at rates of 10-40 g AI/ha.

TABLE 2. Weeds Controlled (93-100%) by AC 322,140 at 45-50 g AI/ha in Rice 2-15 Days After Transplanting

Perennials	Annuals
Cyperus serotinus	*Cyperus difformis*
Eleocharis congesta	*Elatine triandra*
Eleocharis kuroguwai	*Lindernia annua*
Sagittaria pygmaea	*Lindernia procumbens*
Sagittaria trifolia	*Monochoria vaginalis*
Scirpus juncoides	*Rotala indica*

Field trials - cereals

AC 322,140 has been extensively field tested in European cereals. At rates of 25-50 g AI/ha, AC 322,140 controlled a number of important broadleaf weeds (TABLE 3), with excellent tolerance in winter wheat, winter barley, and durum wheat at rates up to 150 g AI/ha. Spring barley showed good tolerance to pre-emergence applications, and slight to moderate injury from post-emergence applications.

TABLE 3. Weeds Controlled (85-100%) by AC 322,140 at 25-50 g AI/ha in Cereals

Pre-emergence	Fall Post-emergence	Spring Post-emergence
Anagallis arvensis	*Sinapis arvensis*	*Anagallis arvensis*
Capsella bursa-pastoris	*Brassica napus*	*Sinapis arvensis*
Fumaria officinalis	*Capsella bursa-pastoris*	*Brassica napus*
Matricaria spp.	*Fumaria officinalis*	*Capsella bursa-pastoris*
Mercurialis annua	*Matricaria spp.*	*Fumaria officinalis*
Picris echioides	*Papaver rhoeas*	*Galium aparine*
Veronica persica	*Veronica hederifolia*	*Matricaria spp.*
		Polygonum convolvulus
		Sonchus spp.

AC 322,140 showed good activity when applied post-emergence on *Galium aparine*. Post-emergence application timing in winter cereals had an important effect on control of *Galium aparine*, with more consistent control at a lower rate from spring applications than from fall applications, and better control still from late spring applications compared to early spring applications. When applied post-emergence in the spring with a crop oil used as an adjuvant, excellent, consistent control of *Galium aparine* was achieved with 25 g/ha of AC 322,140, the lowest rate tested. When applied post-emergence in the fall with non-ionic surfactant 75-100 g/ha of AC 322,140 was required for good control of this species.

METABOLISM AND UPTAKE

The crop selectivity of AC 322,140 is due to rapid metabolic detoxification of the herbicide in the shoots of wheat, barley and rice (Rodaway, *et al.*, 1993). Metabolic detoxification mainly involves hydrolysis of the urea bridge resulting in inactive compounds. This differs from the reported metabolism (Beyer *et al.*, 1988) of bensulfuron-methyl in rice, which occurs by demethylation of the pyrimidine ring 6-methoxy substituent to yield the 6-hydroxy derivative. Phytotoxicity to weeds is a result of increased absorption, translocation and lower metabolic activity in the roots and shoots of susceptible weeds.

SOIL BEHAVIOR

Freundlich absorption/desorption isotherms (Jury *et al.*, 1987) were obtained using the batch-slurry method under a variety of conditions and then were used to determine an average K_{OC} value for AC 322,140 (Rodaway, *et al.*, 1993). When three Japanese paddy soils were used, AC 322,140 was found to have a K_{OC} value of 2.0 m^3/kg, which indicates that the compound binds moderately tightly to soil when compared to other pesticides (TABLE 4). Pesticides with this K_{OC} would not be expected to pose a threat to groundwater by leaching from soil.

TABLE 4. K_{OC} Values for Selected Pesticides

Chemical	K_{OC} (m^3/kg)
Dicamba	0.002
2,4-D	0.02
Alachlor	0.12
Atrazine	0.16
Linuron	0.86
AC 322,140	2.0
Methyl parathion	5.1
Parathion	11.0
Chlordane	30.8
Trifluralin	73.0

CONCLUSIONS

In summary, the following characteristics of AC 322,140 can be highlighted:

- Member of a unique new class of AHAS inhibitors, the sulfamoylureas.
- Control of a wide range of economically important dicotyledonous and sedge weeds.
- Excellent selectivity in rice, wheat and barley.
- Metabolism *via* urea bridge hydrolysis producing inactive compounds.
- Tight binding to soil, and low potential for leaching.
- Very favorable toxicological profile.

AC 322,140 is expected to provide farmers with excellent weed control with a low level of environmental risk.

ACKNOWLEDGMENTS

The authors wish to acknowledge the American Cyanamid Global Phase II field research staff for their conduct of the field evaluations.

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KIH-6127, A NEW SELECTIVE HERBICIDE TO CONTROL BARNYARDGRASS IN RICE.

R. HANAI, K. KAWANO, S. SHIGEMATSU

Kumiai Chemical Industry Co., Ltd., Life Science Research Institute, Kikugawa, Ogasa, Shizuoka, Japan

M. TAMARU

K - I Chemical Research Institute Co., Ltd., Fukude, Iwata, Shizuoka, Japan

ABSTRACT

KIH-6127, methyl 2-[(4,6-dimethoxypyrimidin-2-yl)oxy]-6-[1-(methoxyimino)ethyl] benzoate, is a new selective herbicide with outstanding efficacy on barnyardgrass (*Echinochloa* spp.) in paddy rice (*Oryza sativa*). This compound inhibits acetolactate synthase in plants. KIH-6127 at rates of 30-90 g AI/ha provides excellent control of *Echinochloa* spp. from pre-emergence to the four leaf stage. The use rate of KIH-6127 is extremely low in comparison with the recommend rate of molinate and thiobencarb. KIH-6127 shows excellent selectivity on all rice varieties, and thus can be applied at any growth stage of rice. KIH-6127 can be used alone or mixture with other rice herbicides such as bensulfuron-methyl. Preliminary toxicological and environmental fate studies show favorable results.

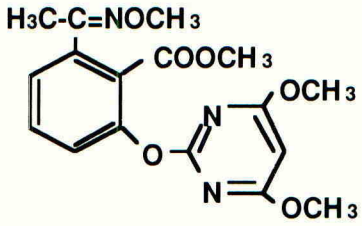
INTRODUCTION

Echinochloa spp. is one of the most important weeds in paddy rice fields in the world. Its seed-setting and germination percentage are so high that its control is very difficult. It is one of most competitive weeds in rice. Infestation of *Echinochloa* spp. results in severe yield loss and quality reduction in rice cultivation. Therefore, rice growers look forward to the introduction of a herbicide which has long lasting efficacy and wide application window for control of *Echinochloa* spp.

KIH-6127 is a novel herbicide introduced by Kumiai Chemical Industry Co., Ltd. This compound has a specific effectiveness against barnyardgrass during a wide range of growth stages from pre- to late post-emergence with an excellent crop safety in rice. Since 1990, many field trials of KIH-6127 and its combination with bensulfuron-methyl have been conducted in water seeded and transplanted rice. In this paper results refer to the control of *Echinochloa* spp. in greenhouse tests and field trials in Japan.

CHEMICAL AND PHYSICAL PROPERTIES

Chemical formula :



Chemical name (IUPAC)	: Methyl 2-[(4,6-dimethoxypyrimidin-2-yl)oxy]-6-[1-(methoxyimino) ethyl]benzoate
Code number	: KIH-6127, KUH-920
Empirical formula	: $C_{17}H_{19}N_3O_6$
Molecular weight	: 361.36
Appearance	: pale yellow grain
Melting point	: 105 ˚C (technical)
Vapour pressure	: 6.243 x 10^{-6} Pa at 25˚C
Solubility	: 11.8 mg/l in water at 20.4 ˚C 4.36 g/100ml in methanol at 20 ˚C

TOXICOLOGY OF TECHNICAL MATERIAL

Acute toxicity	: Oral LD50 rat	> 5000 mg/kg
	Dermal LD50 rat	> 2000 mg/kg
Acute fish toxicity	: Carp LC50	> 10 ppm
	Daphnia carinata	> 10 ppm
Irritation	: Skin, rabbit	slight irritant
	Eye, rabbit	slight irritant
Mutagenicity	: Ames Test, negative	
Teratogenicity	: Non-teratogenic, rat and rabbit.	
Subchronic toxicity	: No effect level, rat : 500 ppm	

MODE OF ACTION

KIH-6127 inhibits the plant enzyme acetolactate synthase thereby blocking branched chain amino acid biosynthesis, like pyrithiobac (KIH-2031), sulfonylurea and imidazolinone herbicides, according to *in vitro* studies using the method of Ray (1984).

FORMULATION

KIH-6127 was formulated as a 10% wettable powder for greenhouse tests and as a 0.1% granule for field trials.

APPLICATION WINDOW

KIH-6127 at rates of 30 - 90 g AI/ha has provided excellent control from pre-emergence to the 4 leaf stage of *Echinochloa* spp. (Table 1).

TABLE 1. Efficacy of KIH-6127 on different growth stages of *Echinochloa oryzicola* in greenhouse test (Kikugawa, Japan)

Compound	Rate	Herbicidal activity			
	(g AI/ha)	Pre-emergence	2 leaf stage	3 leaf stage	4 leaf stage
KIH-6127	90	10	10	10	10
	60	10	10	10	9
	30	10	10	9	8

Herbicidal activity was evaluated visually by a 0 to 10 rating system : 0 = no effect, 10 = complete kill.

RESIDUAL ACTIVITY

KIH-6127 has provided excellent control on *Echinochloa oryzicola* until 50 days after treatment. Residual activity of KIH-6127 at 30 g AI/ha was superior to thiobencarb at 3000 g AI/ha under flooded condition in greenhouse (Figure 1).

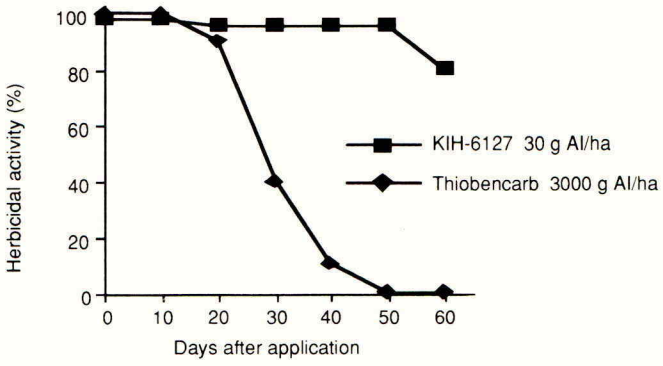


Figure 1. Residual activity of KIH-6127 against *Echinochloa oryzicola*

CROP SAFETY

Phytotoxicity of KIH-6127 at pre-emergence, 2 and 3 leaf stage of rice under flooded condition was very slight at 30 - 90 g AI/ha. Particularly, crop safety of KIH-6127 was superior in pre-emergence treatment to other rice herbicides targeted *Echinochloa* spp. such as molinate (Figure 2).

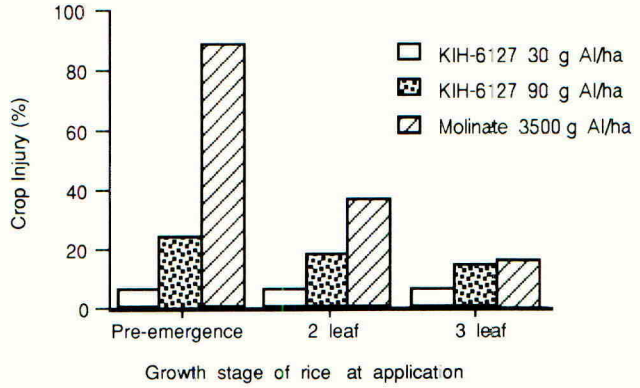


Figure 2. Crop safety of KIH-6127 at pre-emergence, 2 and 3 leaf stage of water seeded rice(*Oryza sativa* : cv Kosihikari)

KIH-6127 has showed an excellent safety on all 11 varieties tested of water seeded rice. There was no observed significant difference in susceptibility to KIH-6127 among rice varieties tested (Table 2).

TABLE 2 Phytotoxicity of KIH-6127 on rice varieties

Varieties (Country)		Crop injury (%)					
	Rate (g AI/ha)	30		90		120	
	Assessed DAA*	20	50	20	50	20	50
M-9 (U.S.A.)		0	0	2	0	5	0
Calrose (U.S.A.)		0	0	1	0	3	0
California pearl (U.S.A.)		0	0	5	0	11	1
Mars (U.S.A.)		0	0	3	0	7	0
Le mont (U.S.A.)		0	0	2	0	7	0
L-202 (U.S.A.)		0	0	1	0	3	0
Mingoro (Dominica)		0	0	1	0	2	0
Rome (Italy)		0	0	4	0	7	0
PR-109 (India)		0	0	0	0	2	0
BG90-2 (Sri Lanka)		0	0	2	0	4	0
Kosihikari (Japan)		0	0	4	0	9	0

Growth stage of rice at application : 3.5 - 4 leaf stage

* DAA = days after application

FIELD TRIALS

KIH-6127 alone and in mixture with bensulfuron-methyl provided good to excellent control of *Echinochloa* spp. from pre-emergence to the 4 leaf stage. However, the mixture with bensulfuron-methyl was superior to KIH-6127 alone in efficacy at the 3 and 4 leaf stage applications. KIH-6127 did not affect the efficacy of bensulfuron-methyl against sedge and broadleaf weeds (Table 3).

Table 3. Crop injury and herbicidal activity of KIH-6127 alone and in combination with bensulfuron-methyl on different growth stages of *Echinochloa* spp. in field trials (Kikugawa, Japan)

Compound	Rate	Crop injury (30 DAA*)				Herbicidal activity (30 DAA*)			
	(g AI/ha)	*Oriza sativa* : cv. Kosihikari				*Echinochloa* spp.			
		5 DAT**	14 DAT	17 DAT	22 DAT	pre-emergence	2 leaf	3 leaf	4 leaf
KIH-6127	90	0	0	0	0	10	10	10	10
	60	0	0	0	0	10	10	10	9
	30	0	0	0	0	10	10	8	7
KIH-6127 + bensulfuron-methyl	30 + 51	0	0	0	0	10	10	10	10

* DAA = days after application
** DAT = days after transplanting
Crop injury and herbicidal activity were evaluated visually by a 0 to 10 rating system : 0 = no effect, 10 = complete kill.

CONCLUSIONS

1. KIH-6127 can be used in a wide application window from pre-emergence to 4 leaf stage of *Echinochloa* spp. in the paddy field.

2. KIH-6127 has a sufficient residual activity in one application for the control of *Echinochloa* spp..

3. KIH-6127 can be applied at any growth stage of rice in water seeded and transplanted rice.

4. KIH-6127 is used at very low rates of 30 - 90 g AI/ha.

5. Toxicity of KIH-6127 against animals and fish is low.

ACKNOWLEDGEMENTS

We would like to acknowledge the work of our colleagues in Kumiai Life Science Research Institute and K - I Chemical Research Institute.

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CGA 152'005 - A NEW HERBICIDE FOR CONTROL OF BROADLEAVED WEEDS IN EUROPEAN MAIZE

M. SCHULTE, K. KREUZ, N. NELGEN, M. HUDETZ, W. MEYER

CIBA-GEIGY Limited, Plant Protection Division - Research and Development Weed Control, CH-4002 Basle, Switzerland

ABSTRACT

CGA 152'005 is a new sulfonylurea herbicide developed by Ciba-Geigy Ltd., Basle, Switzerland, for control of broadleaved weeds in maize. Amongst known sulfonylureas for use in maize, CGA 152'005 is the most selective for broad spectrum dicotyledonous weed control. CGA 152'005 is rapidly metabolized in maize shoots; its half-life has been determined to be approximately 2 hours in excised leaves. No differential varietal crop tolerance to maize hybrids has been identified. CGA 152'005 offers foliar and residual activity on the major weed flora of maize. Post-emergent, at rates between 10 and 30 g AI/ha it controls important genera such as *Amaranthus, Abutilon, Chenopodium, Polygonum, Rumex* and *Stellaria*. CGA 152'005 is mixture-compatible with various maize herbicides. For broad spectrum weed control including *Solanum nigrum* in Europe, mixtures containing CGA 152'005 and other broadleaf herbicides have been developed. CGA 152'005 and its mixtures can be used for post-emergent broadleaved weed control following a pre-emergent applied grass herbicide such as metolachlor and its mixtures with triazines, or for targeted control of triazine-resistant weed species.

INTRODUCTION

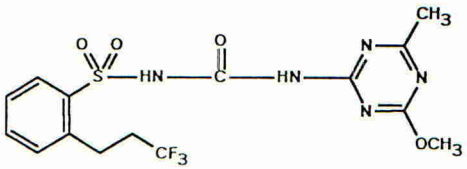
For post-emergent control of both triazine-sensitive and -resistant broadleaved weeds in European maize, various alternative solutions are available. Disadvantages of the current herbicides, however, are either their limited application timing flexibility, their lack of residual activity and thus the need for repeat applications, their limited weed spectrum at maize-selective rates or use restrictions due to ecological concerns. CGA 152'005 is a new herbicide from the chemical class of sulfonylureas for selective broad spectrum dicotyledonous weed control in maize. It offers both foliar as well as residual activity and great application timing flexibility, combined with a minimum impact on the environment.

BASIC PROPERTIES OF THE ACTIVE INGREDIENT

Chemical and physical properties

Chemical name (IUPAC)	1-(4-methoxy-6-methyl-triazin-2-yl)-3-[2-(-3,3,3-trifluoropropyl)-phenylsulfonyl]-urea
Common name	*(not yet released)*

Chemical structure



Appearance	crystalline, colourless, odourless
Molecular weight	419.4
Melting point	155 °C with decomposition
Vapour pressure	< 3.5 x 10^{-6} Pa @ 25 °C
Water solubility	4000 mg/l @ pH 6.8/25 °C
Partition coefficient octanol/water	log P_{OW} = -0.21 @ pH 6.9

Toxicology and ecotoxicology

Acute oral LD_{50} (rat)	986 mg/kg
Acute dermal LD_{50} (rabbit)	> 2000 mg/kg
Acute LC_{50} inhalation (rat, 4 hrs)	> 5000 mg/m^3

Not irritating to skin; not irritating to eyes (rabbit)
Not sensitizing to skin (guinea pig)
Not-mutagenic in five tests
Not-teratogenic in rat and rabbit
Non-toxic to fish, daphnia, earthworms, bees, birds
Very low bioaccumulation potential

BIOLOGICAL PROFILE

Uptake and mode of action

CGA 152'005 is absorbed via both the foliage and the plant roots. Studies using tomato and *Aegopodium podagraria* plants suggest phloem translocation of CGA 152'005 into meristematic tissues after foliar uptake.

The mode of action of CGA 152'005 is - as is the case for other sulfonylurea herbicides - the inhibition of the enzyme acetolactate synthase. After application, growth of susceptible species ceases rapidly, followed by discolouration of leaves; death takes one to three weeks to occur.

Crop tolerance

Basis for the excellent maize tolerance of CGA 152'005 is, similar to other sulfonylureas (Brown, 1990), rapid plant-specific metabolism rather than differential uptake and translocation. 24 hours after foliar application of [^{14}C]CGA 152'005 to seven-day old maize seedlings, less than 2% of the absorbed ^{14}C is recovered as unmetabolized parent herbicide (Table 1). Excised leaves of maize seedlings metabolized a pulse of [^{14}C]CGA 152'005, fed via transpiration stream, with a half-life of 1 to 2.5 hours; this is significantly shorter than the metabolic half-life reported for other commercial sulfonylurea herbicides in maize (Palm *et al.*, 1989).

No differential varietal tolerance response on CGA 152'005 has been identified on any maize hybrid. Certain inbred lines for seed production may show reduced tolerance. Varietal tolerance of CGA 152'005 on inbred lines, pop corn and sweet corn is the subject of ongoing investigations.

Crop selectivity of sulfonylurea herbicides can be impaired by the use of certain insecticides, with soil-applied terbufos being the most significant in maize. This interaction has been reported to occur through inhibition of herbicide metabolism in maize shoots by terbufos and other organophosphate insecticides (Kreuz & Fonné-Pfister, 1992). As shown in Table 1, the at-planting incorporation of terbufos (at the recommended rate of the commercial formulation COUNTER® 2G) in the potting substrate, followed by [^{14}C]CGA 152'005 foliar application to seven-day old maize plants did not result in any appreciable increase of the herbicides parent residues as compared to plants grown in the absence of insecticide. No increase of the metabolic half-life of CGA 152'005 could be detected in excised leaves of maize seedlings planted in a terbufos containing substrate. These findings are confirmed by field observations in Europe, suggesting no enhanced risk of maize injury if a post-emergent use of CGA 152'005 at locally recommended rates follows a soil application of terbufos.

TABLE 1. Effect of a soil-applied organophosphate insecticide on the metabolism of CGA 152'005 in maize. Hybrid cultivar "Blizzard".

Treatment	Uptake [ng/shoot]	Herbicide parent in shoot** [ng/shoot]	*[% of Uptake]*
CGA 152'005 foliar* (1672 ng/shoot)	1540 +/- 24	24 +/- 5	*1.6*
Terbufos at planting followed by CGA 152'005 foliar*	1507 +/- 40	31 +/- 6	*2.1*
Primisulfuron foliar* (1108 ng/shoot)	787 +/- 124	166 +/- 19	*21.1*
Terbufos at planting followed by primisulfuron foliar*	791 +/- 101	445 +/- 72	*56.3*

* Seven days after planting ** 24 hours after treatment

Weed control

CGA 152'005 controls the major broadleaved weed flora of maize. The weed control performance of CGA 152'005 in Europe was investigated in Austria, Germany, France, Italy, Switzerland and Spain since 1988. Important European maize weeds controlled by post-emergent application at 10-30 g AI/ha rates are shown in Table 2. The foliar activity of CGA 152'005 is greater than its soil activity; the success of a pre-emergence use of the compound depends strongly on the soil adsorptivity and moisture conditions after application. The addition of surfactants significantly improves the foliar activity of CGA 152'005, indicating an enhanced uptake. Best results were achieved with non-ionic wetting agents and silicone-based adjuvants.

Less susceptible European weeds not controlled by the CGA 152'005 rates projected for post-emergent use in maize are: grasses, *Cyperus* spp., *Galium aparine*, *Solanum nigrum* and *Veronica persica*.

TABLE 2. European key weeds in maize controlled > 85% by CGA 152'005 post-emergent at various rates, including non-ionic wetting agent 0.1% V/V. Averaged field results from Switzerland, Germany, France, Italy, Austria and Spain 1988-1992.

10 g AI/ha	20 g AI/ha	30 g AI/ha
*Abutilon theophrasti**	*Abutilon theophrasti*	*Abutilon theophrasti*
Amaranthus retroflexus	*Amaranthus retroflexus*	*Amaranthus retroflexus*
Bidens tripartita	*Bidens tripartita*	*Bidens tripartita*
		*Calystegia sepium**
Capsella bursa-pastoris	*Capsella bursa-pastoris*	*Capsella bursa-pastoris*
	*Chenopodium album**	*Chenopodium album*
Chenopodium hybridum	*Chenopodium hybridum*	*Chenopodium hybridum*
Chenopodium polysperm.	*Chenopodium polysperm.*	*Chenopodium polysperm.*
Galinsoga ciliata	*Galinsoga ciliata*	*Galinsoga ciliata*
Galinsoga parviflora	*Galinsoga parviflora*	*Galinsoga parviflora*
	Lamium purpureum	*Lamium purpureum*
	Lapsana communis	*Lapsana communis*
Matricaria chamomilla	*Matricaria chamomilla*	*Matricaria chamomilla*
Mercurialis annua	*Mercurialis annua*	*Mercurialis annua*
Myosotis arvensis	*Myosotis arvensis*	*Myosotis arvensis*
	Papaver rhoeas	*Papaver rhoeas*
	Polygonum aviculare	*Polygonum aviculare*
Polygonum convolvulus	*Polygonum convolvulus*	*Polygonum convolvulus*
*Polygonum persicaria**	*Polygonum persicaria*	*Polygonum persicaria*
	Rumex obtusifolius	*Rumex obtusifolius*
	Senecio vulgaris	*Senecio vulgaris*
Sinapis arvensis	*Sinapis arvensis*	*Sinapis arvensis*
Stellaria media	*Stellaria media*	*Stellaria media*
	Viola tricolor	*Viola tricolor*
	Xanthium strumarium	*Xanthium strumarium*
Volunteer sunflowers	Volunteer sunflowers	Volunteer sunflowers

* Higher rate or 2nd application may be required if drought occurs or weeds are in advanced stage at application.

Application timing studies revealed a great timing flexibility for CGA 152'005. Best activity is achieved when weeds are entirely emerged and maize is at the 3-4 leaf stage. Late post-emergent application (maize 6-7 leaves) may still achieve good results, if weeds are not in a too advanced stage (e.g. less than 30 cm height or until appearance of 3rd pair of foliage leaves) and maize does not cover the weed plants. For instance, best control of *Chenopodium*, *Abutilon* and *Amaranthus* was achieved at early growth stages after cotyledon stage, between appearance of first and third pair of leaves.

Combinations with other herbicides

For European countries where *Solanum nigrum* is a serious weed problem in maize, broad spectrum broadleaved weed control by one single application can be achieved by mixtures of CGA 152'005 with partners, providing control of this species. Field tests revealed mixture-compatibility of CGA 152'005 to various maize herbicides such as bentazone, terbuthylazine, dicamba, bromoxynil (or of course its octanoate ester or potassium salt), or primisulfuron-methyl. Combined with non-residual herbicides, CGA 152'005 contributes to additonal residual weed control with sufficient duration of activity to avoid repeat applications. Formulated mixtures for broad spectrum dicotyledonous weed control with CGA 152'005 in maize were developed using primisulfuron-methyl and bromoxynil as partners. The weed control performance of these mixtures is very similar as shown in Table 3.

TABLE 3. Control of selected weeds by formulated mixtures CGA 152'005 + bromoxynil (1:20 ratio) and CGA 152'005 + primisulfuron-methyl (5:3 ratio) post-emergent, including non-ionic wetting agent 0.1 % V/V. Field trial results from Switzerland, Germany, France, Italy and Spain 1992.

Weed species	No. of results	CGA 152'005 + bromoxynil 15 + 300 g AI/ha	CGA 152'005 + primisulfuron-methyl 12.5 + 7.5 g AI/ha
Abutilon theophrasti	(3)	*87	*95
Amaranthus retroflexus	(7)	96	92
Chenopodium album	(11)	96	89
Polygonum convolvulus	(3)	100	96
Polygonum lapathifolium	(2)	94	90
Polygonum persicaria	(7)	93	95
Sinapis arvensis	(2)	98	98
Solanum nigrum	(10)	92	92
Stellaria media	(2)	100	100
Veronica persica	(2)	82	84
Viola tricolor	(1)	82	88
Xanthium strumarium	(1)	95	100
Volunteer sunflowers	(1)	100	99

* Averaged percent control (biomass reduction) as compared to untreated check. Visual evaluation about one month after application.

For control of mixed infestations of broadleaved and grass weeds in maize, CGA 152'005 and its mixtures can be used post-emergent following a pre-emergent applied grass herbicide such as metolachlor (and its mixtures with triazines, respectively), or for targeted control of triazine-resistant weed biotypes.

Behaviour in soil

CGA 152'005 in soil dissipates rapidly by mainly degradation. Field studies under practical conditions in European maize revealed soil half-lives between 8 and approxi-

mately 40 days. As usual, the dissipation rate of CGA 152'005 depends on factors such as temperature, soil moisture, soil biomass and soil type.

Similar to other herbicides from the chemical class of sulfonylureas, imidazolinones or phenoxyalkanoic acids, CGA 152'005 exhibits a relatively high mobility potential in the soil in laboratory test systems. Under conditions as they usually occur in the field after spring application in maize, the expression of this potential is, however, strongly reduced by the fast dissipation of the compound.

Rotational crop response

TABLE 4. Sensitivity of various crops to CGA 152'005 in soil under greenhouse and field conditions. European field results from 1989-1993.

Crop	Sensitivity rank in the greenhouse	Occurrence of rotational crop injury in the field at 20-40 g AI/ha
Winter/spring wheat	not susceptible*	none
Winter/spring barley		none
Winter rye		none
Hard wheat		none
Summer oats		none
Triticale		**
Maize		none
Rice (dry sown)		**
Field pea		none
Green bean		**
Potato		**
Oilseed rape		none
Perennial ryegrass		none
Soybean		none
Field bean		none
Egyptian clover		none
Sugar beet		occasionally***
Alfalfa		occasionally***
Sunflower	very susceptible	occasionally***

* At highest applied rate (100 g AI/ha) pre-emergent
** No European field results available
*** Further investigations are necessary

As known for certain other herbicides, rotational crops other than the target crop differ greatly in their sensitivity to residues of CGA 152'005 in the soil. Despite its rapid degradation, very low residue levels in the soil may damage non-target crops due to the high intrinsic activity of CGA 152'005 as compared to conventional maize herbicides. The sensitivity of various crops to CGA 152'005 has been determined in the greenhouse; the ranking by the herbicide concentration in the potting substrate causing no observable crop injury is shown in Table 4. The sensitivity ranking in the greenhouse correlates well to the respective field observations from crop rotations. Key rotational crops following

maize, such as small grain cereals, maize, field grass, peas or beans, did not show any injury if weed control in maize was carried out with 20 or 40 g AI/ha CGA 152'005. Based on the correlation of the crop sensitivity ranking under greenhouse conditions to that observed in field studies, it is likely that for extremely susceptible crops such as sugar beet, sunflower and alfalfa the recropping interval after application of CGA 152'005 in maize could be longer than the usual practice, to achieve maximum safety margins. Studies of the rotational crop safety of CGA 152'005 at practical use rates are ongoing.

CONCLUSION

In Europe, CGA 152'005 offers diverse benefits for the user such as: Management of most dicotyledonous weed problems in maize, in particular where triazine herbicides are no longer effective, maximum crop safety without varietal restrictions on any maize hybrid, foliar activity with great application timing flexibility, sufficient residual activity on annual weed species without need of repetitive applications, low application rates minimizing impacts on the environment, reduced storage costs, safe and easy handling due to formulation as a water-dispersible granule, full rotational flexibility to key crops following the target crop maize, and use without insecticide restrictions. The combination of various features of CGA 152'005 constitutes a new state of the art for selective broadleaved weed control in maize as compared to conventional standard herbicides.

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NOTES

KIH-2023, A NEW POST-EMERGENCE HERBICIDE IN RICE

M. YOKOYAMA, O. WATANABE, K. KAWANO, S. SHIGEMATSU

Kumiai Chemical Industry Co., Ltd., Life Science Research Institute, Kikugawa, Ogasa, Shizuoka, Japan

N. WADA

K - I Chemical Research Institute Co., Ltd., Fukude, Iwata, Shizuoka, Japan

ABSTRACT

KIH-2023, sodium 2, 6-bis [(4, 6-dimethoxypyrimidin-2-yl) oxy] benzoate, is a new post-emergence herbicide for the control of a wide range of weeds with excellent selectivity in direct-seeded rice (*Oryza sativa*). The low rate of 15 - 45 g AI/ha + surfactant has provided outstanding efficacy on *Echinochloa* spp. and can be applied from 1 to 7 leaf stage of the weed. Also it can control other important weeds including *Brachiaria* spp., *Cyperus* spp., *Scirpus* spp., *Polygonum* spp., *Sagittaria* spp., *Commelina* spp., and *Sesbania exaltata*. Rice exhibits good tolerance to KIH-2023 at these rates. Results of preliminary toxicological and environmental fate studies are favorable.

INTRODUCTION

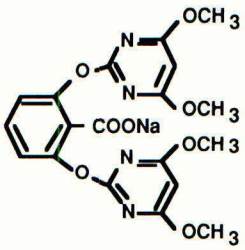
In direct-seeded rice, some annual and perennial weeds, especially *Echinochloa* spp. frequently escape from application of existing commercial rice herbicides which usually require relatively high rates of use. Rice growers continue to look for flexible and low-rate post-emergence herbicide to optimize crop yield.

KIH-2023 is a novel post-emergence herbicide discovered and being developed by Kumiai Chemical Industry Co., Ltd. for use in direct-seeded rice. This herbicide exhibits excellent efficacy against a wide range of economically important grass and broadleaf weeds in rice (Wada *et al.*, 1990). The greatest commercial potential for KIH-2023 is for the selective control of *Echinochloa* spp. at low use rates with a wide application window.

This paper describes the chemical and physical properties of KIH-2023 and summarises results from greenhouse tests and world-wide field trials which have been conducted over four years.

CHEMICAL AND PHYSICAL PROPERTIES

Structure :



Common name	: None given yet
Chemical name (IUPAC)	: Sodium 2, 6-bis [(4, 6-dimethoxypyrimidin-2-yl) oxy] benzoate
Chemical formula	: $C_{19}H_{17}N_4NaO_8$
Appearance	: White powder
Molecular weight	: 452.4
Melting point	: 228.0 ˚C (decomposition)
Water solubility	: 733 g/l (at 25 ˚C)
Vapour pressure	: 5.05 x 10^{-9} Pa (at 25˚C)

TOXICOLOGY

Acute oral LD50 (rat)	: 4111 mg/kg (male), 2635 mg/kg (female)	
Acute dermal LD50 (rat)	: > 2000 mg/kg	
Inhalation LC50 (rat)	: > 4.48 ml/l	
Acute fish toxicity LC50	: Bluegill sunfish : >100 ppm	
Rainbow trout	: > 100 ppm	
Eye irritation (rabbit)	: Slightly irritant	
Skin irritation (rabbit)	: Not irritant	
Ames mutagenicity	: Negative	
Teratogenicity (rat, rabbit)	: Non-teratogenic	
Subchronic toxicity	: No effect level,	rat : 100 ppm (male), 1000 ppm (female)
		dog : 100 mg/kg/day (male, female)

MODE OF ACTION

KIH-2023, like sulfonylurea and imidazolinone herbicides, inhibits the plant enzyme, acetolactate synthase, thereby blocking branched chain amino acid biosynthesis. KIH-2023 controls sensitive species with cessation of growth followed by chlorosis, necrosis, and plant death.

SELECTIVITY

KIH-2023 has high selectivity between rice and *Echinochloa oryzicola* by foliar application under dry-seeded conditions, suggesting that KIH-2023 can be used against a wide range of growth stages of *Echinochloa* spp. without rice crop injury (Figure 1).

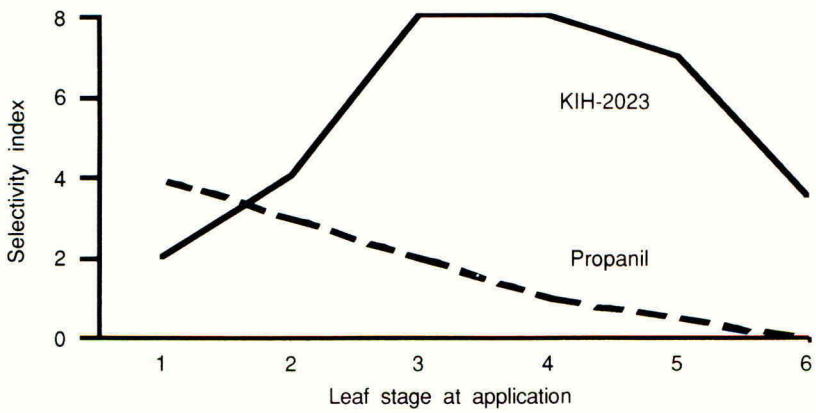


Figure 1. Selectivity of KIH-2023 between rice and *Echinochloa oryzicola* by foliar application under dry-seeded conditions in the greenhouse. (Kikugawa, Japan, 1989)

$$\text{Selectivity index} = \frac{\text{Rate required for 10\% rice injury}}{\text{Rate required for 90\% Echinochloa oryzicola control}}$$

APPLICATION WINDOW

The efficacy of KIH-2023 is relatively independent of growth stage of *Echinochloa oryzicola* and KIH-2023 at 30 g AI/ha is more effective for controlling 3 - 7 leaf stage *Echinochloa oryzicola* than propanil at 3000 g AI/ha (Figure 2).

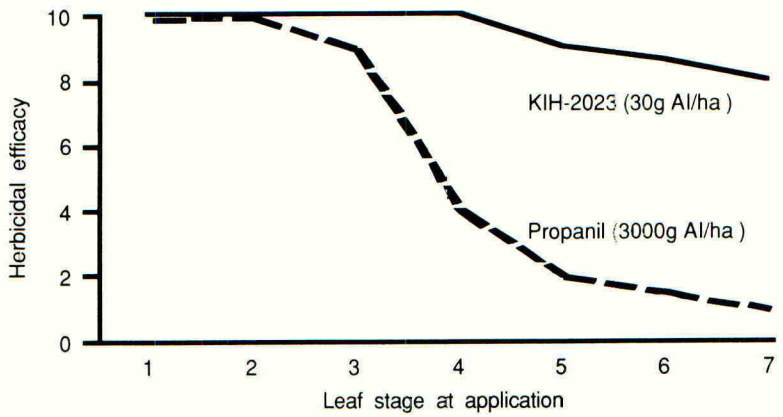


Figure 2. Efficacy of KIH-2023 on different growth stages of *Echinochloa oryzicola* by foliar application under dry-seeded conditions in the greenhouse. (Kikugawa, Japan, 1989)

Herbicidal efficacy (visual assessment) : 0 = no effect - 10 = complete kill

EFFICACY

Adjuvants such as non-ionic surfactants, silicon type adjuvants or crop oil concentrates play an important role in enhancing activity and achieving the consistent performance of KIH-2023.

The results of world-wide field trials carried out from 1988 through 1992 indicate that KIH-2023, when applied post-emergence at the rate of 15 - 45 g AI/ha, shows excellent control of a wide range of key weeds in dry-seeded, water-seeded and rainfed rice.

The following weeds have been controlled by KIH-2023 (at 15 - 45 g AI/ha) :

Grasses

Brachiaria plantaginea
Echinochloa oryzicola
Echinochloa colonum
Leptochloa fascicularis
Sorghum halepense (seedling)

Sedges

Cyperus difformis
Cyperus iria
Fimbristylis annua
Fimbristylis miliacea
Scirpus juncoides
Scirpus mucronatus

Broadleaf weeds

Aeschynomene sensitiva
Ammannia coccinea

Bacopa spp.
Commelina communis
Commelina diffusa
Damasonium minus
Eclipta prostrata
Jussiaea linifolia
Limnocharis flava
Mimulus orbicularis
Mollugo pentaphylla
Monochoria vaginalis
Polygonum lapathifolium
Portulaca oleracea
Rumex japonicus
Sagittaria guayanensis
Sagittaria pygmaea
Sesbania exaltata
Sphenoclea zeylanica

All post-emergence trials have been conducted with commercial rates of a surfactant or a non-phytotoxic petroleum-based spray oil.

KIH-2023 is most effective when applied to vigorously growing *Echinochloa* spp. at the 3 - 6 leaf stage. At the same time, KIH-2023 can control other important weeds in direct-seeded rice.

CONCLUSIONS

The wide spectrum of activity and the wide application window by KIH-2023 allow it to be considered a basic tool for weed control in direct-seeded rice. KIH-2023 offers some new options to rice farmers for the following reasons :

1. KIH-2023 can be used in a wide application window from 1 - 7 leaf stage of *Echinochloa* spp..

2. KIH-2023 is effective against many species of weeds, such as annual and perennial grasses, sedges and broadleaf weeds, and propanil-resistant *Echinochloa* spp..

3. KIH-2023 shows a stable efficacy under a wide range of soil and climatic conditions.

4. KIH-2023 can be applied in mixture or in close sequence with other pesticides including carbamate or organophosphorous insecticides.

5. KIH-2023 is used at the very low rates of 15 - 45 g AI/ha.

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Wada, N., *et al.* (1990) Pyrimidine Derivatives and Herbicidal Method and Compositions, *United States Patent*, 4,906,285.

METOBENZURON - A NEW UREA HERBICIDE FOR BROAD-LEAVED WEED CONTROL IN CORN

T. MORIMOTO, N. KIHARA, I. HASHIMOTO, T. ISHIDA, T. ISHITOKU

Specialty Chemicals, Chemical Synthesis Laboratories,
Mitsui Petrochemical Industries, Ltd.,
Waki-cho, Kuga-gun, Yamaguchi, 740 Japan.

T. TAKEMATSU

Plant Science Research Institute,
Naka-Okamoto, Kawachi, Tochigi, 329-11 Japan.

ABSTRACT

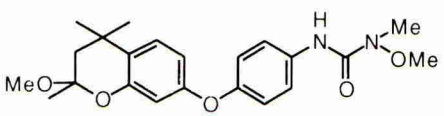
Metobenzuron, (+/-)-1-methoxy-3-[4-(2-methoxy-2,4,4-trimethylchroman-7-yloxy)phenyl]-1-methylurea, developed by Mitsui Petrochemical Industries, Ltd. (Mitsui code : UMP-488) is a new selective early post-emergence herbicide for the control of various broad-leaved weeds in corn (*Zea mays*). The compound shows remarkable inhibiting activity in photosynthesis. In greenhouse and field tests, metobenzuron alone at 125-250 g AI/ha shows promising control of various early leaved-stage weeds, such as *Abutilon theophrasti*, *Xanthium strumarium*, *Chenopodium album*, *Ambrosia elatior*, *Ipomoea hederacea* and *Amaranthus retroflexus*, including triazine-resistant weeds. By combination with 2,4-D and/or bromoxynil, metobenzuron shows excellent control of numerous broad-leaved weeds in field trials in the USA and France. This paper discloses the product chemistry, the physico-chemical properties, the toxicological profile and biological activities of metobenzuron.

INTRODUCTION

Metobenzuron is a new urea herbicide discovered by Mitsui Petrochemical Industries, Ltd. It is being developed for the early post-emergence control of various annual broad-leaved weeds in corn (*Zea mays*). Field trials in corn have been conducted mainly in the USA and France since 1987. This paper describes the properties of metobenzuron and summarizes results from greenhouse and field trials over several years.

CHEMICAL AND PHYSICAL PROPERTIES

Structure:



Chemical name:	(+/-)-1-methoxy-3-[4-(2-methoxy-2,4,4-trimethylchroman-7-yloxy)phenyl]-1-methylurea
Common name:	metobenzuron (proposed by ISO)
Code number:	UMP-488
Chemical formula:	$C_{22}H_{28}N_2O_5$
Molecular weight:	400.5

Melting point:	101.0-102.5℃
Appearance:	White powder
Vapor pressure:	1.86×10^{-5} mm Hg (22.3℃)
Solubility in water:	0.4 mg/l (25℃)

TOXICOLOGY

Acute Oral LD_{50} (rat):	Male, Female > 10,000 mg/kg
(mouse):	Male, Female > 10,000 mg/kg
Acute Dermal LD_{50} (rabbit):	Male, Female > 2,000 mg/kg
Eye Irritancy (rabbit):	slightly irritant
Skin Irritancy (rabbit):	none
Mutagenicity (Ames test):	negative

ENVIRONMENTAL FATE IN SOIL AND METABOLISM IN CORN

Environmental study of metobenzuron shows low mobility and a short half-life in soil. Metobenzuron is also easily metabolized in corn (Table 1).

TABLE 1. Mobility and metabolism of metobenzuron.

Mobility in sandy loam	TLC Rf = 0.10		
Decomposition on soil	T1/2 = 4 hr (under irradiation) T1/2 = 8 hr (in the dark)		
Metabolism in corn (concentration ppm)	after application	→ harvest time	
		fodder	grain
	254	0.012	<0.006

MODE OF ACTION

The main action of metobenzuron is inhibition of photosynthesis. Laboratory experiments using thylakoid membranes showed that the inhibition activity of metobenzuron is about 20-100 times higher than those of standard herbicides which have the same mode of action (Table 2).

TABLE 2. Photosynthesis inhibition activities.

Compound	Inhibition Activity IC_{50} (μM)
metobenzuron	0.004
atrazine	0.398
metribuzin	0.251
diuron	0.079
linuron	0.126

MATERIALS AND METHODS

Greenhouse Tests

Greenhouse testing was carried out using a silt loam soil. Metobenzuron alone or mixed with other herbicides was applied to corn and weeds, which were at the 2-5 leaf stage. Visual assessments were made 3-20 days after treatment.

Field Trials

Field trials have been conducted in the USA and Europe since 1987. All applications were made at early post-emergence of the corn, using hand carried CO_2 charged plot sprayers with flat fan nozzles at a pressure of 200-350 kPa in a spray volume of 180-400 l/ha. Visual assessments were made 7-30 days after treatment.

RESULTS

Greenhouse Tests

Metobenzuron at 125-250 g AI/ha gave promising control of many annual broad-leaved weeds tested in the greenhouse with early post-emergence application. It has been shown that adjuvants such as crop oil concentrates are necessary to achieve acceptable performance. Corn showed a good tolerance to metobenzuron up to 250 g AI/ha (Table 3). Combinations with other herbicides have also been investigated. The combinations of metobenzuron+2,4-D with addition of surfactant and urea ammonium nitrate showed high efficacy against the main broad-leaved weeds and high selectivity on corn. In this case, the rate of metobenzuron could be reduced to 47 g AI/ha in greenhouse tests (Table 3).

TABLE 3. Efficacy of metobenzuron alone, or in combination with 2,4-D in greenhouse.

Species	Phytotoxicity Score*		
	metobenzuron**		metobenzuron+2,4-DA***
Rate (g AI/ha)	125	250	47+75
Abutilon theophrasti	8	9	9
Xanthium strumarium	9	9	10
Chenopodium album	10	10	10
Ambrosia elatior	8	9	9
Ipomoea hederacea	8	9	10
Amaranthus retroflexus	10	10	10
Corn	0	1	0

* Phytotoxicity score : 0 = no effect, 10 = completely killed.

** 0.3% of Agri-Dex (crop oil concentrate, Helena Chemical Company) was added.

*** 2,4-DA is 2,4-D dimethylamine salt. 0.1% of Genapol X060 (surfactant, Hoechst) and 3% of urea ammonium nitrate were added.

Field Trials in USA

Table 4 shows the mean percentage of weed control and crop injury achieved by early post-emergence application of metobenzuron in the USA (1989-1990). Metobenzuron treatment resulted in good broad-leaved weed control and selectivity.

TABLE 4. Efficacy and crop injury of metobenzuron* alone in corn (mean of trials, USA, 1989-1990).

Species	% control metobenzuron		
Rate (g AI/ha)	63	125	250
Abutilon theophrasti	37	72	78
Chenopodium album	77	83	97
Amaranthus viridis	78	92	96
Datura stramonium	96	98	99
Corn	0	2	8

* 0.3% of Agri-Dex was added.

Field trials on the combination of metobenzuron+2,4-D were also carried out in 1991-1992, and the combination demonstrated excellent efficacy on important broad-leaved weeds with early post-emergence application (Table 5).

TABLE 5. Efficacy and crop injury of the combination of metobenzuron+2,4-D* in corn (mean of trials, USA, 1991-1992).

Species	% control			
	metobenzuron+2,4-DA		metobenzuron+2,4-DLV**	
Rate (g AI/ha)	95+150	125+125	95+150	125+125
Abutilon theophrasti	94	94	95	88
Xanthium strumarium	99	86	98	95
Chenopodium album	99	99	99	99
Ambrosia elatior	80	83	83	99
Ipomoea hederacea	95	87	91	93
Corn	0	1	0	0

* 0.1% of Genapol X060 and 3% of urea ammonium nitrate were added.

** 2,4-D isooctyl ester.

Field Trials in France

Table 6 shows the mean percentage weed control achieved by early post-emergence application of metobenzuron in France (1989-1990). Metobenzuron treatment showed good control of triazine resistant broad-leaved weeds.

TABLE 6. Efficacy of metobenzuron* on triazine-resistant weeds in corn (mean of trials, France, 1989-1990).

Species	% control			
	metobenzuron			atrazine
Rate (g AI/ha)	63	125	250	3000
Amaranthus viridis	95	95	95	32
Chenopodium album	83	90	95	33
Solanum nigrum	95	100	100	37
Polygonum aviculare	80	83	93	0
Corn	0	3	5	0

* 0.3% of Agri-Dex was added.

Field trials on the combinations of metobenzuron+2,4-D and metobenzuron+2,4-D+bromoxynil were also carried out in France (1992), and the combinations demonstrated good to excellent efficacy on important broad-leaved weeds following early post-emergence application (Table 7).

TABLE 7. Efficacy and crop injury of the combinations of metobenzuron+2,4-D* and metobenzuron+2,4-D+bromoxynil** in corn (mean of trials, France, 1992).

Species	% control	
	metobenzuron+2,4-DA	metobenzuron+2,4-DA+bromoxynil
Rate (g AI/ha)	125+125	125+125+125
Chenopodium album	95	99
Solanum nigrum	100	99
Amaranthus viridis	100	100
Polygonum persicaria	87	77
Polygonum aviculare	83	58
Polygonum convolvulus	93	98
Corn	5	2

* 0.1% of Genapol X060 was added.
** 0.05% of Genapol X060 was added.

CONCLUSIONS

Metobenzuron is a new urea herbicide, which has high potential for controlling important broad-leaved weeds following early post-emergence application in corn at a rate of 125 - 250 g AI/ha. Combination with 2,4-D increases efficacy and widens the spectrum at a low rate of metobenzuron such as 95 g AI/ha. Metobenzuron alone and in combination with 2,4-D has also shown high activity against troublesome triazine-resistant weeds in Europe. Metobenzuron and its combination with 2,4-D have been expected to play an important role in controlling a wide range of broad-leaved weeds including triazine-resistant weeds in corn.

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TWO NOVEL CELL DIVISION TYPE PGRs

ZHENG-MING LI*, LI-XIN QIAO, ZHONG-REN ZHAO

Elemento-Organic Chemistry Institute, Nankai University, Tianjin, China 300071

ABSTRACT

28 new N-2-thiazolyl and thiazolinyl-N'-substituted phenylureas were synthesized by the reaction of the heterocycles and substituted phenyl isocyanates. Their cytokinin activity was examined by cucumber cotyledon expansion bioassay in which N-thiazolyl and N-thiazolinyl-N'-(m- chlorophenyl) - ureas showed good PGR activity in comparison to kinetin. The structure / activity relationship are discussed.

INTRODUCTION

Thiazoles and thiazolines were firstly investigated as pharmaceutical candidates in the 1950s for example penicillin. Since the 1960s, some fungicides, such as benzothizaoles, have been reported (Ram Laken et al.,1986; E. Sidova et al., 1985). 2-(p-chloroanilino)-thiazoline, which was formerly an antibiotic, was also investigated as a fungicide (Han 1991). On the other hand, some pesticides with ureido structure have been reported (Showa Denko). In the task of developing new pesticides, it seems reasonable to consider thiazoline and ureido moieties together in designing novel structures. Although some N-thiazolinyl-N'-substituted phenylureas (I) have already been synthesized

(Najer et al., 1963), their bioactivity has not been fully described. Here we carried out a systematic synthesis of thiazolyl and thiazolinyl- phenyl ureas and their cytokinin activty was examined.

METHODS

Chemicals

In previous study, N-2-thiazolinyl-N'-substituted phenyl-ureas were prepared by rearrangement method (H. Najer et al., 1961, 1963). In our study, all of the urea compounds were prepared by the addition reaction of substituted phenyl isocyanate and 2-amino-thiazoles or thiazolines. These were all identified by elemental analysis, ^{1}H-NMR and IR. The compounds prepared were shown in Figure 1 and 2.

Cucumber cotyledon expansion bioassay

The cucumber cotyledon expansion bioassay was done as previously described (Zhao et al., 1988, 1992). The cucumber (Cucumis sativus L. cv. Jinyan No. 4) seeds were sown on 0.7 % agar and grown in the dark at 26°C. Cotyledons of even size were excised from 3 day old seedlings for the weight promotion test. The results were recorded in Table 1.

Test for the content of chlorophyll

Ten cotyledons were taken from each petri dish and cut into 3 mm pieces. Then the pieces were put into a flask containing 40 ml absolute ethanol. After overnight extraction, absorbance was measured at 645 nm and 663 nm. The content of total chlorophyll was calculated according to the Arnon's formula (Arnon, 1949) (Table 2).

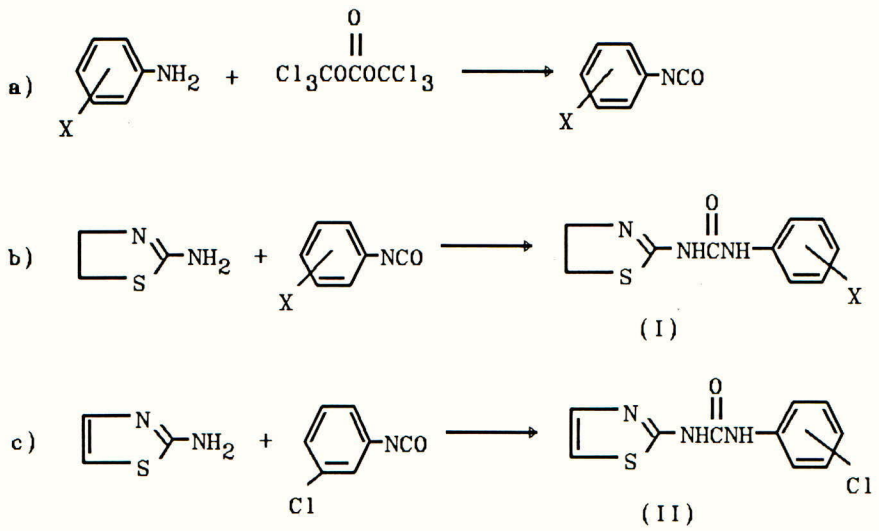
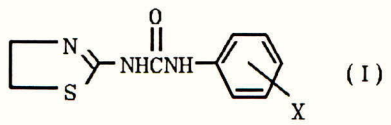


Fig 1. Synthetic methods for the phenylureas



Ia	2-Cl	Ib	3-Cl	Ic	4-Cl
Id	4-F	Ie	2-Me	If	2,4-Cl_2
Ig	2,3-Cl_2	Ih	3,4-Cl_2	Ii	2-Br
Ij	4-Br	Ik	2-NO_2	Il	4-NO_2
Im	2,4-$(NO_2)_2$	In	2,4,5-Cl_3		

Fig 2. Substituent X in different compounds (Ia-In) in general formula (I)

Table 1. A comparison of new urea compounds with kinetin

compd.	conc.	fresh wt of cotyldeon (mg/10 cotyledons)	(i/d%)
Ia	10	496	19.5
Ib	10	674*	62.4
Ic	10	401	-3.3
Id	10	476	14.6
Ie	10	471	13.4
If	10	416	0
Ig	10	471	13.4
Ih	10	501	20.7
Ii	10	497	19.7
Ij	10	427	2.8
Ik	10	484	16.6
Il	10	440	6.0
Im	10	452	8.9
In	10	444	6.9
Kinetin	10	695	67.4
control (distilled water)		415	

i/d% = % increase/decrease over untreated
Kinetin supplied by Shanghai Biochemistry Institute, Chinese Academy of Sciences, m.p. 265.8-266.9°C (sealed tube). Merck Index (1989) m.p. 266-267°C (sealed tube).

Table 2. A comparison of the PGR activity of Ib, II with Kinetin by the cucumber cotyledon expansion bioassay

compd.	conc. (ppm)	fresh wt of cotyledons		total chlorophyll content of cotyledons			
		mg/ 10 ctl.	i/d %	mg/ 10 ctl.	i/d %	mg/g fresh wt	i/d %
Ib	1	503	13.6	0.344	22.5	0.683	7.5
	10	688	55.3	0.459	63.4	0.677	4.8
	100	753	70.0	0.401	43.0	0.532	-16.2
II	1	573	29.3	0.387	37.9	0.675	6.1
	10	761	71.7	0.442	57.3	0.580	-8.6
	100	869	96.3	0.465	65.7	0.524	-17.4
kinetin	1	543	26.6	0.369	30.6	0.675	6.1
	10	719	62.3	0.414	47.3	0.575	-9.5
	100	831	87.7	0.355	26.6	0.427	-32.8
control (distilled water)		443		0.282		0.636	

note: ctl. = cotyledons
i/d = % increase/decrease over untreated

3. RESULTS AND DISCUSSION

There has not been yet any report on the PGR activity for N-2-thiazolyl (thiazolinyl)-N- substituted phenylureas. This is the first time that their cytokinin activity has been reported. Table 1 shows that the 2,4-dichloro derivative has an activity of zero while others showed different activity. The activity sequence for the N-2-thiazolinyl-N'-chloro-substituted phenyl ureas is as following: 3 > 2 > 4. N-2-thiazolinyl-N'-(m-chlorophenyl)-urea (Ib) is the most active compound and in some respects (such as chlorophyll increase) exceeds kinetin.

By modifying the ureido structure, we found that N-2-thiazolyl-N'- (chlorophenyl)-urea (II) shows higher activity than N-2-thiazolinyl - N'-(m-chlorophenyl)-urea (Ib). The content of chlorophyll was measured and the comparative results were listed in Table 2. In terms of the weight of cotyledons, II is superior to kinetin and Ib is slightly inferior to kinetin. In terms of the content of chlorophyll, II is superior to kinetin and Ib is slightly inferior to kinetin. The wider range of concentration effects of II provides larger tolerance. Based on the results mentioned above, we have applied for a patent (Li et al., 1992) of the cytokinin activity of compound Ib and II and their analogues.

ACKNOWLEDGEMENTS

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