Department of Environmental Chemistry

Year-round production of large quantities of high-quality agricultural products is associated with repeated heavy loading of farmland with fertilizers, pesticides, and livestock wastes. This, in turn, leads to air, water, and soil pollution with substances such as pesticides, nitrates, and heavy metals. With increasing combustion of refuse, lethal dioxins are being released to the environment. The Department of Environmental Chemistry has a mandate for food security and ecosystem conservation against a number of farm chemicals from the 3 broad standpoints of "risk assessment", "risk reduction", and "environment remediation".

The Department consists of 3 research groups and 1 team corresponding to the chemicals targeted, namely: 1) a group researching organic chemical compounds such as farm chemicals; 2) a group researching heavy metals (in particular, cadmium); 3) a group researching nutritional salts, such as nitrogen and phosphate; and 4) the dioxin research team. Each group has a leader and several research units. The major research fields of each group are described below.

Organochemicals Group: Pesticides play a vital role in food security and will remain indispensable unless more effective and less risky replacements can be developed. There is much concern about the ecotoxicity of pesticides in air, water, and soil from farmlands. This group is responsible mainly for the development of innovative and sophisticated technologies for studying the influence of pesticides on the environment and how to decrease the amounts of chemicals used. Major research topics are: 1) the dynamics of pesticides in soils, water, and the atmosphere; 2) risk assessment of pesticides in aquatic organisms such as algae, aquatic midges, and medaka fish (killifish); 3) development of environmentally friendly crop protection systems, and 4) development of technologies for the bioremediation of recalcitrant organic compounds, involving a) molecular genetics and genetic diversification of bacteria that degrade chlorobenzoates, PCBs, and 2,4-D, b) *in situ* bioremediation of soils contaminated with recalcitrant organic compounds, and c) risk assessment of recombinant bacteria.

Heavy Metal Group: The Codex Committee, established jointly by FAO and WHO, has been developing a new international safety standard for cadmium in foods to minimize its human intake. Under such circumstances, it is a matter of urgency that we elucidate the behavior of heavy metals in soils and the mechanism of their absorption by crops, and that we develop technologies to suppress hazardous metal absorption by crops. The Heavy Metal Group has 3 ongoing research projects: 1) evaluation of heavy metal loadings in arable soils and elucidation of the mechanisms of their absorption by crops; 2) elucidation of the chemical forms of heavy metals in soils and development of technologies for suppression of their absorption by crops; and 3) determination of differences in the abilities of various staple crops to absorb heavy metals.

Water Quality and Solute Dynamics Group: Recently, public concern has risen over the contamination of various river basins and lakes by nutrient solutes such as nitrate nitrogen and phosphate. Since the implementation of new regulations against NO_3-N and NO_2-N contamination began in 1999, a number of agricultural activities have been placed under strict surveillance to ensure that NO_3-N and NO_2-N levels in groundwater do not exceed the critical concentration of 10 ppm. There is an urgent need to formulate an effective solution for this problem. There are 3 ongoing projects in this group: 1) study of the dynamics of nitrate nitrogen and other nutrient solutes in soils and small- and medium-sized watersheds; 2) development of methods for monitoring levels of nutrient solutes in medium-sized river basins; and 3) evaluation of methods for enhancing the denitrification capabilities of natural mass flows and development of technologies for alleviating negative loadings of nutrient solutes.

Dioxin Dynamics Team: Contamination of agricultural products with dioxins has become a serious concern for both consumers and producers. There is an urgent need for the production of dioxin-free agricultural products. In this regard, there are 2 ongoing projects: 1) study of the dynamics of dioxins in crops and farmland, and 2) development of technologies for the physico-chemical and biological decomposition of dioxins.

1) Organochemicals Group

The missions of this group are to assess and reduce the environmental risk caused by application of pesticides and persistent organic pollutants (POPs) in agro-ecosystems and to develop bioremediation techniques to restore environments contaminated with recalcitrant organic chemicals.

In FY 2004, the group studied the following major research subjects: 1) risk assessment of pesticides in aquatic organisms; 2) multimedia modeling to predict the fate of POPs; 3) mechanism of induction of systemic acquired resistance in plants by some organic chemicals; 4) molecular genetics and molecular ecology of bacteria that degrade chlorobenzoates and 2,4-D in soils; 5) *in*

situ bioremediation of pesticide-contaminated soil by using charcoal enriched with degrading bacterial consortia.

Sixteen original research papers were published this FY. In September 2004, the group organized the fourth Seminar on Organic Chemicals Studies: Mechanisms of POP Persistence in Soil and the Technology of Risk Reduction and Principles and Utilization of Soil Adsorption, and the 21st Research Meeting on Pesticides: Environmental Risk Management of Drift in Pesticide Application.

The **Environmental Pesticide Assessment Unit** developed a system for the indoor breeding of the caddisfly (*Cheumatopsyche brevilineata*) to assess the effects of pesticides on aquatic invertebrates in inland water ecosystems (Topic 1). The caddisfly was selected as an insect representative of those in Japanese rivers—that is, as a key species. By bioassays using native algal species such as diatoms, the Unit clarified the effects of herbicides on algal production and observed a wide range of susceptibility to certain herbicides in the middle reach of the river, where many paddy fields are located. For POPs, the Unit is developing a prototype multimedia model to clarify how POPs are emitted and diffused from Asian regions.

The **Pesticide Mitigation Unit** met the challenge to develop an alternative chemical—a resistance inducer or "plant vaccine"—for the control of fungal plant diseases, which currently relies largely on the use of ordinary fungicides. As an alternative chemical, the Unit selected acibenzolar-*S*-methyl (ASM) and studied the mechanism of long-lasting induction of systemic resistance in plants. In ASM-pretreated cucumber plants, the gene encoding callose (β-1,3-glucan) synthase was highly expressed after fungal attack, resulting in physical blockage of pathogen penetration and development. The longevity of systemic resistance induced by ASM was well demonstrated in cucumber under greenhouse condition. Fewer spray applications of ASM than of ordinary fungicides still maintained a high level of protection against powdery and downy mildews on cucumber. In addition, the Unit used the sequence difference in a β-tubulin gene to identify the *Fusarium* species that cause head blight on cereals. The PCR-Luminex system was successfully applied for this purpose. Furthermore, biological monitoring studies showed that isolates resistant to strobilurin fungicides are widely distributed in Japan, irrespective of the usage history of this class of fungicides. Molecular characterization of the resistance mechanism is under investigation at present. By designing a pair of forward and reverse primers related to carboxylesterase, the Unit also developed a simple gene diagnosis method for specific qualitative detection of strains of cotton aphids resistant to organophosphorus (OP) insecticides (Topic 2).

The **Applied Soil Microbiology Unit** studied the mechanisms regulating expression of the chlorobenzoate and 2,4-D degradative genes of various soil isolates and the chitinase genes of *Streptomyces* spp.. A study of chimeric mutants of chlorocatechol 1,2-dioxygenases revealed several amino acid residues responsible for the differences in substrate specificities. The Unit also analyzed the characteristics of plasmids with 2,4-D degrading genes. 3-chlorobenzoate (3CB)-degrading bacteria whose concentrations increased in a forest soil after addition of 3CB were detected by a culture-independent method, polymerase chain reaction - denaturing gradient gel electrophoresis (PCR-DGGE). Furthermore, to develop *in situ* bioremediation of pesticide-contaminated soil, bacterial consortia decomposing both quintozene (PCNB) and simazine were constructed in charcoal. The simazine-decomposing bacterial consortia were found to be composed of *Arthrobacter* spp., *Bradyrhizobium japonicum,* and a novel strain of β-*Proteobacteria.*

Topic 1: System for indoor breeding of caddisfly (*Cheumatopsyche brevilineata*) for ecological toxicity assessment

Japan's current pesticide registration system requires us to perform a set of acute toxicity examinations in 3 species—fish (e.g. carp or medaka, as high-order consumer), water flea (as primary consumer), and green algae (as producer)—to assess the ecotoxicity of a pesticide in inland water ecosystems. "Inland water" covers extended areas from small streams to huge lakes, and there is also an immense variety of species. Taking into consideration the fact that susceptibility to pesticides differs among species, a predicted effect concentration is determined by dividing the toxicity values (EC_{50} and LC_{50}) in water flea and fish by a safety margin (= 10). To determine a more accurate safety margin, further ecotoxicity studies are needed using aquatic organisms which are important and representative of certain inland water ecosystems, in addition to the three species of test organisms.

In inland water ecosystems, the dominant primary consumer species are water flea and aquatic insects such as mayfly and caddisfly. Such ecosystems around paddy fields in Japan are characterized by numerous areas of running water, such as the rivers or irrigation canals that join the paddy fields. In many cases water fleas live in static waters, such as lakes. Running water is not their common habitat. In running water regions, the dominant species are mayfly and caddisfly. Because the mayfly has a complicated life cycle, it is considered very difficult to

breed. For this reason we selected the caddisfly instead as a target species. From among the caddisfly species we selected those of the family Hydropsychidae (especially *Cheumatopsyche brevilineata*), because they commonly live in the small rivers or irrigation canals directly connected to paddy fields throughout Japan. *C. brevilineata* was therefore suitable for evaluating the ecotoxicity of paddy pesticides. We developed an indoor breeding system to establish an acute toxicity study of caddisfly larvae.

The species of *C. brevilineata*, called as "Kogata-shima-tobikera" in Japan, inhabits gravel bottom of rivers or canals. The food of the larvae is mainly algae or organic matter flowing down the river. The larvae make silken thread nets, which they use to catch their food. Maturing larvae (fifth instar) make cocoons underwater. Hatched adults resemble moths, and the females lay egg masses on stony surfaces underwater. Under natural conditions the caddisfly has a bivoltine life cycle and overwinters in the larval stage (Fig. l).

We developed a system for indoor breeding of *C. brevilineata* (Fig. 2), using the following devices. First, we found that larvae could not survive with a shortage of oxygen in the water, so we maintained water flow in the

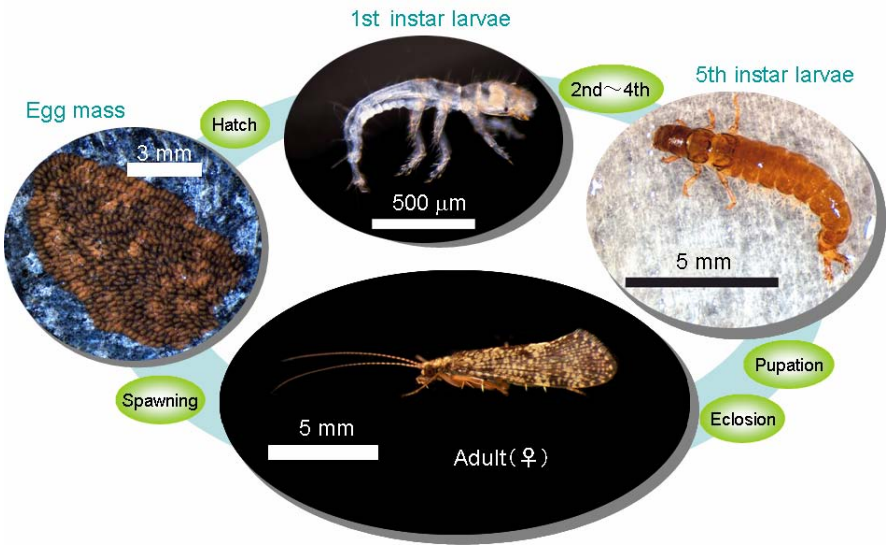

Fig.1 Life cycle of the caddisfly (*Cheumatopsyche brevilineata*).

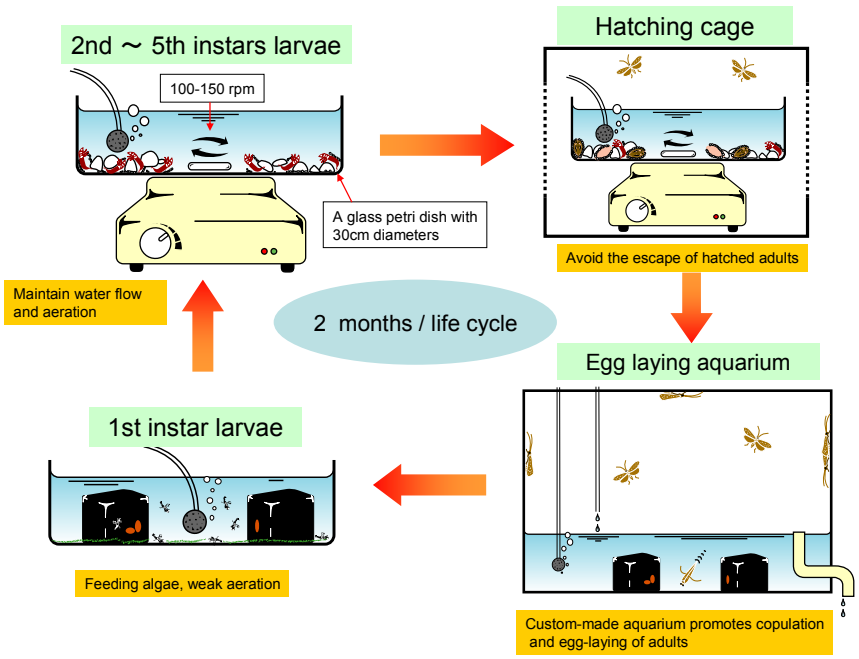

Fig.2 The indoor breeding systems of *C. brevilineata.*

vessel with a magnetic stirrer and aerated the water with an air pump. Second, when we fed only commercial flake fish food, the first instar larvae did not grow well and had a high mortality rate, so we improved their survival rate by feeding algae instead. Larvae at the second or greater instar were able to grow well when fed the commercial fish food. Third, we also devised a custom-made cage covering the breeding vessel to prevent the escape of hatched adults. Finally, we developed a custom-made aquarium to promote copulation and egg-laying of hatched adults. We filled the aquarium with water to a depth of about 8 cm and placed some cubic black rocks (about 5 cm square) in the water as sites for egg-laying.

We succeeded in breeding 4 or 5 generations a year of *C. brevilineata*, with 500 to 1000 individuals per generation, and were thus able to continuously supply first instar larvae for acute toxicity testing of pesticides. Further studies will be needed, but we expect to be able to develop chronic toxicity methods of *C. brevilineata* and apply our breeding system to other species of caddisfly. (K. Ohtsu and A. Yokoyama)

Topic 2: **Specific detection of strains of cotton aphid resistant to organophosphorus insecticides using nucleotide sequence differences in a detoxification enzyme gene**

Since the appearance of chemically synthetic insecticides in the 1940s, many examples of insecticide resistance in various species of agricultural and hygienic insect pests have been reported, and resistance has now been reported in over 500 species. The development and spread of insecticide resistance on arable lands have led to the application of excessive amounts of insecticides and have increased the negative impact on surrounding environments. Accurate and timely information on the status of resistant populations in the field is needed for mitigation of the risk associated with this resistance. Thus far, bioassays and/or measurement of detoxification enzyme activity have been the main methods used to determine resistance status. These traditional methods, however, are time consuming and costly and have high labor requirements, and the data obtained are of low accuracy. In addition, these methods cannot detect potential risky element carriers, namely insects heterozygous for recessive resistance genes or "revertants" whose resistance is regulated by methylation in the promoter region of the detoxification enzyme gene. Therefore, gene diagnosis would be the most effective way to accurately determine the status of, and trends in, the development of resistance.

The cotton aphid *Aphis gossypii* Glover is a serious pest of many crops and has developed resistance to various classes of insecticides. Overproduction of a detoxification enzyme, carboxylesterase (CE), had been proved to be responsible for OP insecticide resistance in *A. gossypii*. However, the nature of the differences in the CE between susceptible and resistant aphids has not been examined. We have been attempting to determine the CE-cDNA sequences in several strains of *A. gossypii*, with the aim of developing a gene diagnosis method for OP insecticide resistance.

We compared the CE-cDNA sequences in 1 super-susceptible (SS), 3 susceptible (S), and 4 resistant (R) strains reared in our laboratory. The LD_{50} values for fenitrothion, an OP insecticide, were 5.70, 22.4, or 113 ppm for the SS, S or R strains, respectively. Three different sequences were obtained, i.e. *ss*-, *s*-, and *r*-types (Fig. 3). Each sequence was divided into 2 regions: the amino acid coding region of CE, and the 5′ untranslated region forward of the CE region (Fig. 3). Prominent differences in length and nucleotide order were found in the later 5′ region sequences of these 3 types. SS strains had only the *ss*-type sequence, and S strains had only the *s*-type sequence. However, R strains had both *r*-type and *s*-type sequences. It was interesting to note that *r*-type cDNA was detected by real-time PCR approximately 20 times more frequently than the *s*-type. Therefore, R strains were distinguishable by the *r*-type sequence, and we consider that overexpression of *r*-type mRNA is responsible for resistance.

On the basis of these results, we developed a method

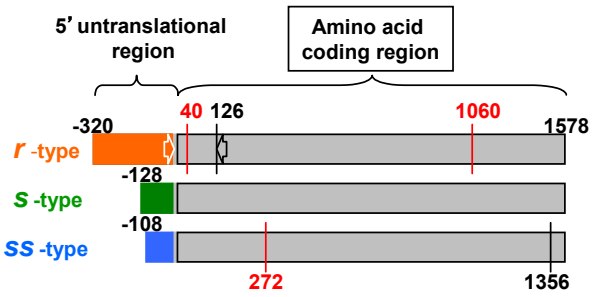

Fig.3 Comparison of the 3 CE-cDNA structures identified from organophosphorus-insecticide resistant, susceptible, and super-susceptible strains of the cotton aphid.

Vertical bars and numbers written on the amino acid coding regions of the *r*-type and *ss*-type indicate sites where nucleotide substitutions were found, in comparison with the *s*-type sequence. (Red and black bars respectively indicate sites with and without amino acid substitutions.) Sequences of the 5' untranslated region without homology among the three types are shown in different colors.
Arrows drawn on the r-type cDNA indicate PCR primers for specific detection of the resistant strains (see Fig. 4).

for the specific qualitative detection of OP insecticide-resistant cotton aphids. We designed a pair of forward and reverse primers related to CE. The forward primer was designed to anneal selectively to the 5′ untranslated region sequence of the *r*-type sequence. The reverse primer was designed to anneal to the amino acid coding region of CE with the *r*-type sequence by putting an *r*-type specific nucleotide site at the 3′ terminus of the primer (Fig. 3). By PCR using this primer pair, we could selectively detect resistant strains (Fig. 4). Unlike real-time PCR, this method does not require expensive apparatus or difficult quantitative processes such as adjustment of template concentrations among all samples and/or preparation of standard genes. Furthermore, this primer pair is effective not only for cDNAs as templates but also for genomic DNAs (Fig. 4). It is extremely useful as a rapid, simple, and accurate way of monitoring the status of resistance development. We are now attempting to improve the method: our targets are to skip the process of cleanup of the PCR templates and to downsize to downsize the analytical method for identifying the resistance with one individual of cotton aphid.. (K. Suzuki)

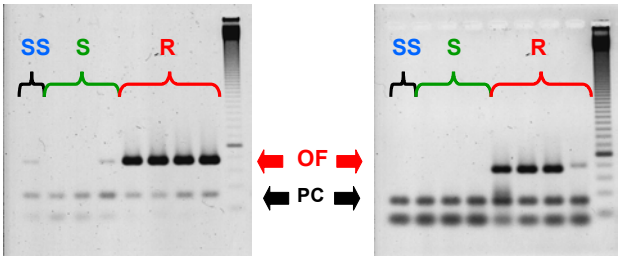

Fig. 4 Specific detection of organophosphorus insecticide- resistant strains of cotton aphid by PCR.

OF: Objective fragment
PC: Positive control (partial fragment of Acyl-CoA dehydrogenase)
R: resistant strains, S: susceptible strains,
SS: super-susceptible strain

2) Heavy Metal Research Group

The mission of the Heavy Metal Research Group is to elucidate the input–output balance of heavy metals such as cadmium (Cd) in arable soils and to clarify the mechanisms by which these metals are absorbed and translocated by paddy rice and soybean.

In FY 2004, the group studied the following 8 subjects: 1) phytoremediation of Cd-contaminated paddy fields by special rice varieties; 2) Cd input from rainfall in the city of Tsukuba; 3) remediation of Cd-contaminated paddy soil by washing with chemicals (see Main Research Results 1); 4) translocation characteristics of Cd absorbed by soybean (Topic 1); 5) effects of paddy field water management on the absorption of Cd by rice; 6) screening of soybean varieties for low Cd uptake and low accumulation in grains; 7) mechanisms of absorption and translocation of Cd in low-Cd-absorbing cultivars of rice (Topic 2) and soybean; and 8) evaluation of heavy metal loading of arable soils by fertilizers.

Topic 1: Uptake and transport of cadmium in hydroponically cultured soybean plants

If we are to effectively reduce the Cd content of seed, it is important that we determine the growth stages of the soybean (*Glycine max* var. Enrei) during which absorbed Cd is most likely to be transferred to the seed. The results of previous soil-pot and field experiments suggest that absorption of Cd before the beginning of the seed stage (R5) causes an increase in the Cd concentration of the seed. However, the details of this were still not clear. Using hydroponically grown soybean, we therefore evaluated the most critical stages of soybean development at which Cd absorbed via the roots was transferred into the seeds. Soybean plants at different growth stages were fed for 48 h with a culture solution containing Cd at 0.01 mg L^{-1}.

The Cd concentration in the seeds at the stage of full maturity (R8) differed depending on the growth stage during which the Cd was absorbed. The concentration of Cd in the seeds was highest when the soybean had absorbed Cd at the full pod stage (R4) through to the full seed stage (R6).

The cumulative Cd uptake by soybean seeds, as calculated from the rate of Cd uptake per day, is shown in Figure 1. Cd uptake during the full pod stage (R4)

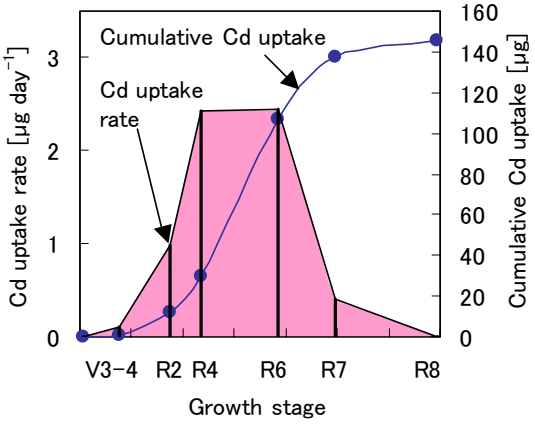

Fig. 1 Cadmium (Cd) uptake rate per day per plant and cumulative Cd uptake in soybean seeds per plant at 5 different growth stages.

through to the full seed stage (R6) accounted for approximately 50% (the largest proportion) of the total Cd content of the seeds. The amount of Cd absorbed during the third- or fourth-node stages (V3–V4) through to the full pod stage (R4) comprised approximately 20% (a minor contribution) of the total seed Cd content (Fig. 1). Therefore, to lower seed Cd content, it is important to reduce Cd absorption via the roots during the full pod stage (R4) through to the full seed stage (R6).

To investigate Cd transfer to the aerial parts, soybean plants to which we had applied Cd at the third- or fourth-node stage (V3–V4) were sampled at different growth stages. We then determined the Cd uptake in various aerial parts (Fig. 2). The absorbed Cd was transferred into the stems, petioles, and leaves until the full bloom stage (R2). At the full seed stage (R6) and the beginning of the maturity stage (R7), the Cd content of the leaves decreased steeply, whereas that in the pods and seeds increased. This result indicated that Cd accumulated in the leaves was translocated to the seeds during the full seed stage (R6) and the beginning of the maturity stage (R7).

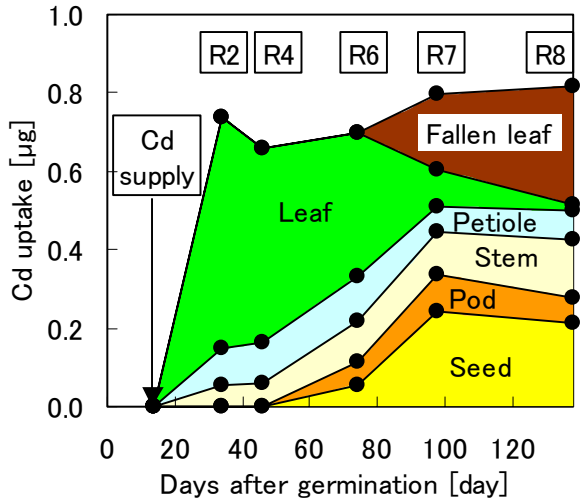

Fig. 2 Distribution of Cd in 6 parts of the soybean plant at 5 different growth stages. For 48 h each soybean plant at the third- or fourth-node stage (V3–V4) absorbed a culture solution containing Cd at 0.01 mg L^{-1}.

These results confirmed the tendency found in field experiments of Cd accumulation by soybean. To reduce Cd concentrations in soybean seeds it is important to develop practical soil management strategies to reduce Cd absorption during the R4 to R6 stages. (A. Kawasaki and H. Oda)

Topic 2: **Locating gene loci related to cadmium concentration in brown rice**

Of the daily foods of the Japanese people, rice is responsible for half the Cd uptake through the diet, and so we need to reduce concentrations of Cd in rice to levels as low as possible. The development of cultivars of brown rice that have low Cd contents is considered to be an effective permanent technology for reducing the uptake of dietary Cd. However, there has never been any research aimed at locating the QTLs (quantitative trait loci) that control the accumulation of Cd in brown rice. We attempted to locate the gene loci associated with Cd accumulation in brown rice by using a novel mapping population consisting of 39 chromosome segment substitution lines (CSSLs) in rice. Our aim was to obtain genetic information useful in the efficient development of cultivars with low Cd concentrations.

The 39 mapping populations, which carried a single chromosome segment of 'Kasalath' (*indica*) in each line overlapping with neighboring segments in a 'Koshihikari' (*japonica*) genetic background, were grown in pots (1/5000 a, four pots per CSSL) filled with Cd-contaminated soil (Gray Lowland soil, 0.1 mol L^{-1} HCl-extracted Cd concentration, 1.8 mg kg^{-1}). To elucidate the differences between 'Koshihikari' and each CSSL in terms of brown rice Cd concentration, we used growing conditions in which Cd was easily absorbed into the plant (water levels were maintained at 60% of field capacity). After grain-ripening, we collected the brown rice and measured its Cd concentration. Differences in grain Cd concentration between each CSSL and the recurrent parent 'Koshihikari' were evaluated by Dunnett's pairwise multiple comparison *t*-test ($P < 0.1$).

The brown rice of 3 CSSLs in which chromosomes 3 (SL-207 and SL-208) and 8 (SL-224) were partly substituted showed significantly lower Cd concentrations than that of 'Koshihikari'. The average Cd concentration of SL-223 was similar to that of SL-224, although the probability level somewhat exceeded the threshold of 0.1. On the other hand, 3 CSSLs in which chromosome 6 was partly substituted had significantly higher grain Cd concentrations than 'Koshihikari' (Fig. 3).

We investigated the phenotypic correlation between the Cd concentrations in the brown rice of the CSSLs and 16 quantitative traits representing agronomic, physiological, and morphological characters, and we found that the brown rice Cd concentration was correlated with 7 of those traits.

On the basis of the graphical genotypes of CSSLs whose brown rice showed significant differences in Cd concentrations relative to that of 'Koshihikari', we mapped the putative QTLs related to grain Cd concentra-

tion on chromosomes 3, 6, and 8 (Fig. 4). The results suggested that these QTLs might be linked to those for the 7 traits that had phenotypic correlations, indicating a pleiotropic effect of the QTLs for grain Cd concentration.

The use of CSSLs with a 'Koshihikari' genetic background could markedly shorten the time needed to breed 'Koshihikari' whose Cd concentration of brown rice is low. Because a 'Kasalath' segment on chromosome 6 carries a QTL related to a high grain Cd trait, it is important not to take in this region when using the effective genes on chromosome 6 in breeding. (S. Ishikawa)

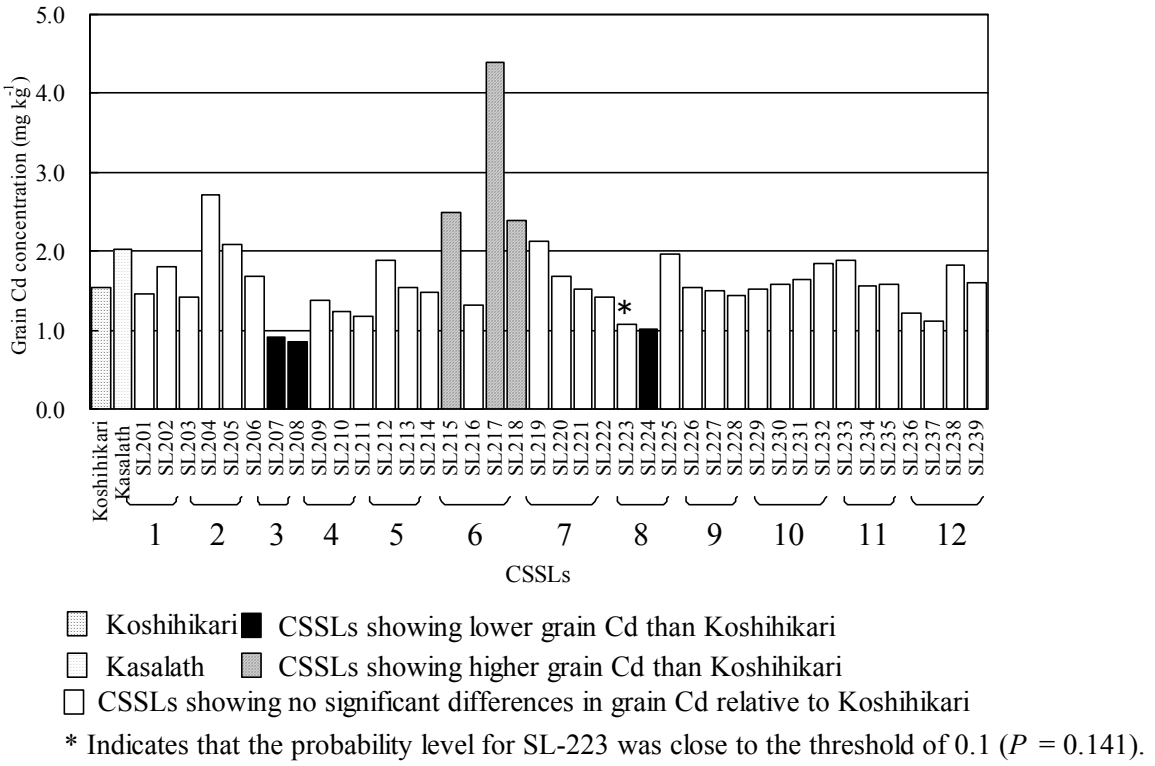

Fig. 3 Grain Cd concentration of CSSLs and their parents. The number under each name indicates the number of the chromosome at which each line was substituted.

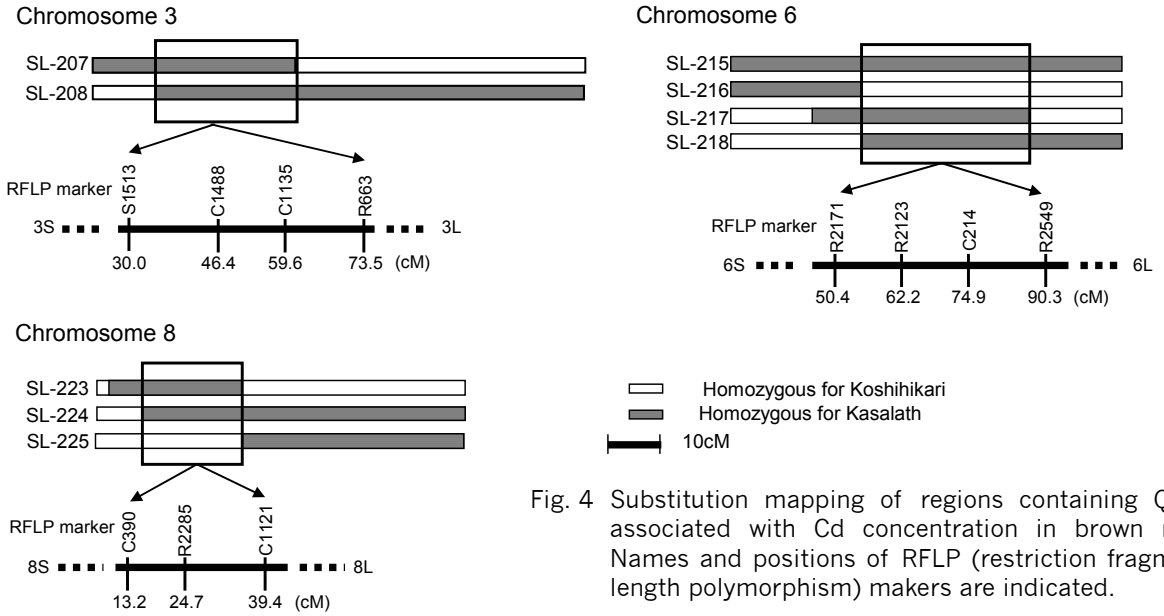

Fig. 4 Substitution mapping of regions containing QTLs associated with Cd concentration in brown rice. Names and positions of RFLP (restriction fragment length polymorphism) makers are indicated.

3) Water Quality and Solute Dynamics Group

The mission of the Water Quality and Solute Dynamics Group is to clarify the dynamics of solutes such as nitrate-nitrogen (NO_3-N) passing through arable lands to water bodies; to develop technologies to monitor loadings of NO_3-N and other pollutants; and to reduce these loads on the environment. We have 4 ongoing projects: 1) elucidation of the mechanisms of solute movement through the soil and below ground; 2) development of monitoring methods for NO_3-N and other pollutants in medium-sized river basins; 3) development of a technology for alleviating the agricultural nitrogen load on the environment by enhancing denitrification; and 4) construction of a model adaptable to medium-sized river basins for prediction of nitrogen load and effluent. In FY 2004, we elucidated the discharge of suspended matter and associated phosphorus to tile drains in a clayey field with subsurface cracks (Topic 1) and also estimated the origin of nitrous oxide in shallow groundwater under upland fields (Topic 2).

Topic 1: **Discharge of suspended matter and associated phosphorus to tile drains in a clayey field with subsurface cracks**

In general, phosphorus is adsorbed and precipitated strongly on soil particles and is insoluble in water, so that it hardly migrates vertically in soil. However, in fields with a clayey subsoil with shrinkage cracks—particularly ex-paddy fields that were used for paddy rice for many years and then recently converted for upland crops—it is likely that soil particles on which phosphorus is immobilized will migrate vertically through the cracks. To elucidate the process of discharge of suspended matter and associated phosphorus to tile drains in a clayey ex-paddy field, we measured the soil water condition, surface runoff, and tile discharge. We also collected water in the surface runoff and the tile drainage to determine the suspended particle (> 0.1 μm) and phosphorus concentrations.

In Niigata Prefecture we set up an experimental field on a Mottled Gley Lowland soil where soybean had been grown on an ex-rice paddy for 9 years and shrinkage cracks had opened in the clayey subsoil. Discharge of water to the tile drains was initiated soon after the pressure potential at the topsoil–plowsole boundary became positive (Fig. 1). The discharge responded quickly to changes in rainfall intensity, and there was no change in the water condition of the subsoil, suggesting that water flow occurred preferentially through the cracks (Fig. 1).

The concentrations of phosphorus in the tile drainage were highest at the beginning of discharge, with a maximum concentration of 1.3 mg-P/L. In tile drainage that had a concentration of phosphorus greater than 0.2 mg-P/L, more than 80% of the phosphorus was in the suspended form. During the period of soybean planting in 2004, about 90% (0.51 kg-P ha^{-1}) of the total discharge of phosphorus (0.57 kg-P ha^{-1}, about 5% of fertilization) to the tile drains was estimated to be in the suspended form. These values exceeded those for the suspended form of phosphorus (0.21 kg-P ha^{-1}) in surface runoff (0.27 kg-P ha^{-1}, about 2% of fertilization) (Fig. 2). These results demonstrate that subsoil cracks can be major pathways for the discharge of suspended soil particles and associated phosphorus from clayey ex-paddy fields. (K. Suzuki)

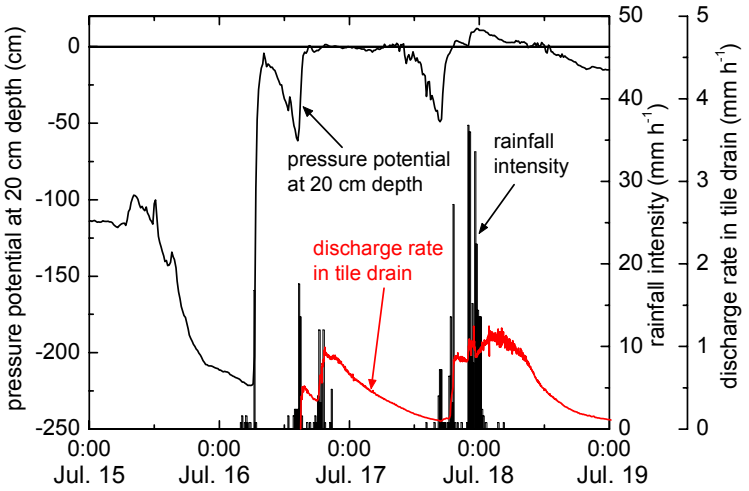

Fig. 1 Rainfall intensity, discharge rate to tile drains, and soil water potential at the topsoil–plowsole boundary during a rainfall event in mid-July in mid-July 2004.

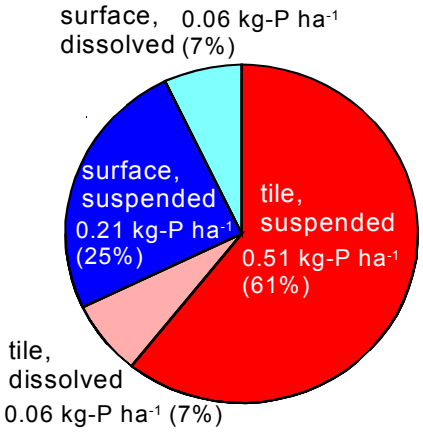

Fig. 2 Estimated discharge of phosphorus from an ex-paddy field during the monitoring period.

Topic 2: **Origin of nitrous oxide in shallow groundwaters under upland fields**

The nitrogen that originates from chemical fertilizers and animal wastes leaches mostly in the NO_3-N form and reaches the shallow groundwater. There, part of the NO_3-N is reduced by denitrifiers to N_2 gas or nitrous oxide (N_2O), which is an intermediate denitrification process. N_2O is often detected in spring waters. However, the denitrification activity and nitrogen dynamics in the groundwater remain to be determined. During a 3-year period of monitoring the quality of the groundwater that flows under upland fields in Ibaraki Prefecture, a high concentration of N_2O (20 to 5630 μg N/mL) was measured in a well. The soil (Cumulic Andosol) had usually received chemical fertilizers annually, and farmers had disposed of livestock excreta in trenches nearby until 1999.

In study well 2, the NO_3-N concentration was high when the water level was high, whereas the NH_4-N concentration was high when the water level was low (Fig. 3a). This suggests that NO_3-N originated from N applied as fertilizer, and the NH_4-N originated from manure. In the vicinity of the well, nitrogen seemed to have originated from different sources such as chemical fertilizers (lower $\delta^{15}N$) and livestock excreta (higher $\delta^{15}N$), which may have mixed together through leaching and horizontal migration. This assumption was supported by the finding that $\delta^{15}N$-TN, which did not include $\delta^{15}N$-N_2O, was low when the water level was high and high when the water level was low (Fig. 3b).

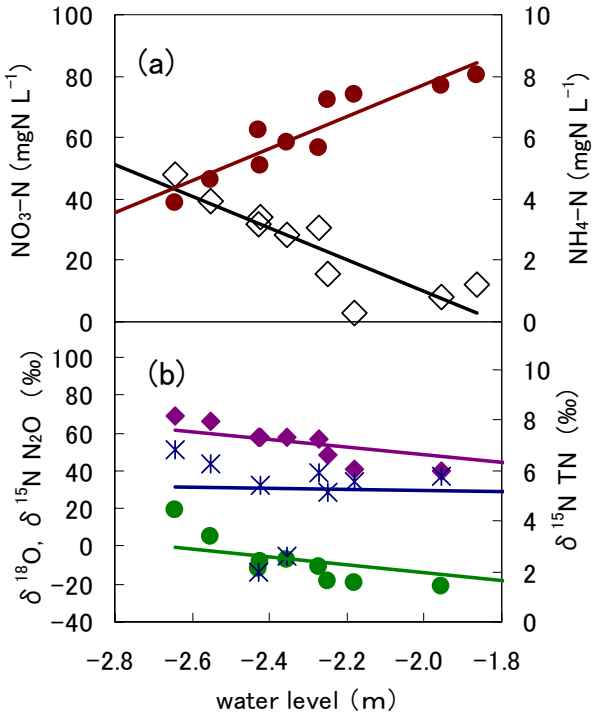

Fig. 3 Relationship between water level and NH_4-(◇) and NO_3- (●) N concentrations, $\delta^{18}O$ (◆), $\delta^{15}N$ of N_2O (●), and $\delta^{15}N$ of TN (＊).

In contrast, $\delta^{15}N$-N_2O was about –20‰ at high-water level and lower than $\delta^{15}N$-TN (Fig. 3b). This suggests that N_2O was produced by nitrification. The ratio of $\delta^{15}N$ to $\delta^{18}O$ of N_2O in the well was about 1 to 1; this ratio was far from 1 to 2, which is the usual value produced by the denitrification process. These results demonstrate that changes in $\delta^{15}N$ and $\delta^{18}O$ do not depend on denitrification in the groundwater, but can be produced by the mixing of "light" N_2O originating from fertilizer with "heavy" N_2O originating from manure. (Y. Nakajima)

4) Dioxin Dynamics Team

The mission of the Dioxin Dynamics Team is to elucidate dioxin dynamics in the agro-environment and to develop technologies to remediate dioxin-contaminated soils. In FY 2004 research was conducted on the following topics: 1) dioxin contamination patterns in crops; 2) temporal changes in concentrations of dioxins accumulated in Japanese paddy soils as a key to pollutant sources (Topic 1); 3) accumulation and behavior of dioxins in arable lands of Japan and Korea (Topic 2); and 4) development of a technology for reducing dioxin outflows from paddy fields.

Topic 1: **Temporal changes in organochlorine pesticide accumulation in Japanese agricultural soils**

Persistent organic pollutants (POPs) are transferred across borders and are accumulated in animals such as polar bears and seals. To prevent or reduce global environmental contamination caused by these compounds through cooperation with countries around the world, the Stockholm Convention on Persistent Organic Pollutants came into effect in May 2004. After the Convention is put into effect, countries will need to use its principles to manage the problems associated with POPs, by such means as prohibition of use, reduction of emissions to the environment, and prediction of future changes in contamination levels. Furthermore, most of the compounds specified as POPs in this agreement are organochlorine pesticides, such as DDT, dieldrin, and chlordane. It is, therefore, important that we understand the temporal trends in organochlorine pesticide concentrations in paddy soils so that we can predict future contamination levels. Paddy soils collected periodically from all over Japan since 1960 are preserved at NIAES. We analyzed these preserved soils to trace changes in organochlorine pesticide concentrations and to elucidate the sources of these contaminants.

The temporal change in organochlorine pesticide

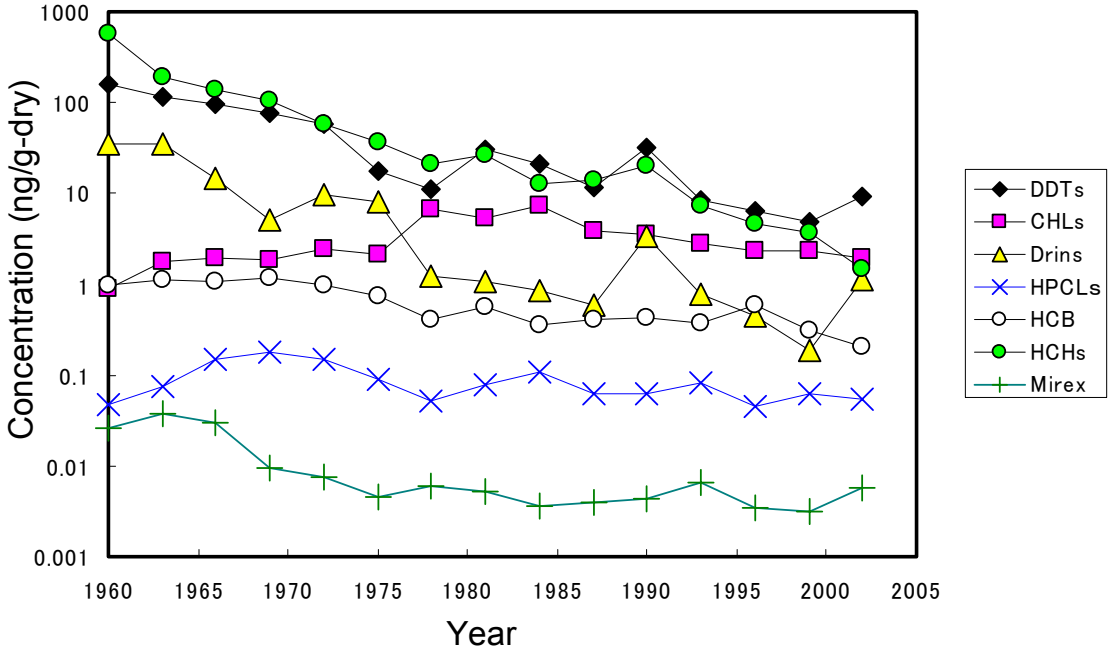

Fig. 1 Temporal change in organochlorine pesticide concentrations (ng/g·dry) in 15 paddy soils from Japan.

concentrations in paddy soils reflected the use of pesticides (Fig. 1). DDT, dieldrin, and HCH were used as insecticides on paddy rice, and their use was banned in the early 1970s; concentrations of DDT, dieldrin, and HCH in paddy soils have decreased drastically from the late the 1960s onward. Chlordane was used until 1986 to control domestic pest insects, but was not used in paddy fields in Japan; concentrations of chlordane in paddy soils increased from the 1970s through to the 1980s, suggesting that chlordane was transported via the atmosphere and/or water during the period when it was used. Concentrations have since been gradually decreasing. Despite the fact that hexachlorobenzene (HCB) was never used as a pesticide in Japan, we detected it in paddy soils throughout the entire period studied. One of the important sources of HCB is impurities of pentachlorophenol (PCP), which was used in large quantities as a paddy herbicide during the 1960s in Japan. Concentrations of HCB in paddy soils increased during the 1960s but have been gradually decreasing since; presumably HCB had accumulated in paddy soils as an impurity in PCP herbicides. Mirex, an insecticide, was never used in Japan, but a small amount of mirex was detected in paddy soils during the entire period, suggesting that mirex was transferred across borders from other countries. (N. Seike)

***Topic 2:* Annual mass balance of dioxins in a Japanese paddy field**

1. Dioxins in Japanese paddy soils

According to the Ministry of the Environment (MOE), the mean dioxin concentration in Japanese paddy soils in 2001 was 46.5 pg-TEQ (toxic equivalent)/g, a value higher than those in upland soils. The mean concentration in NIAES experimental paddy soils was 82 pg-TEQ/g, a little higher than average but within the range of normal paddy soils. The dioxins were derived from impurities in the herbicides chloronitrophen (CNP) and pentachlorophenol (PCP), which were used until they were banned in 1965. We therefore estimated the annual mass balance of dioxins in a Japanese paddy field to predict changes in their concentration in paddy soils. The following factors that influence the concentration of dioxins in paddy soils had to be taken into account: (1) factors that increase dioxin concentrations in soil: atmospheric deposition, input as rice plant parts, and input from irrigation; (2) factors that decrease dioxins in soil: runoff, decomposition, and volatilization. We estimated the values of these factors from our experimental data and references.

2. Factors that increase dioxin levels

Atmospheric deposition: Our data from the NIAES field experiments showed that the dioxin input from the atmosphere was 2.0 ng-TEQ m^{-2} $year^{-1}$.

Input as rice plant parts: The mean dioxin concentration in the leaves and stems of rice was 4.3 pg-TEQ/g (our experimental data). Supposing that the yield was 700 g/m^2, the input to the paddy soils in the form of rice leaves and stems would have been 3.0 ng-TEQ m^{-2} $year^{-1}$.

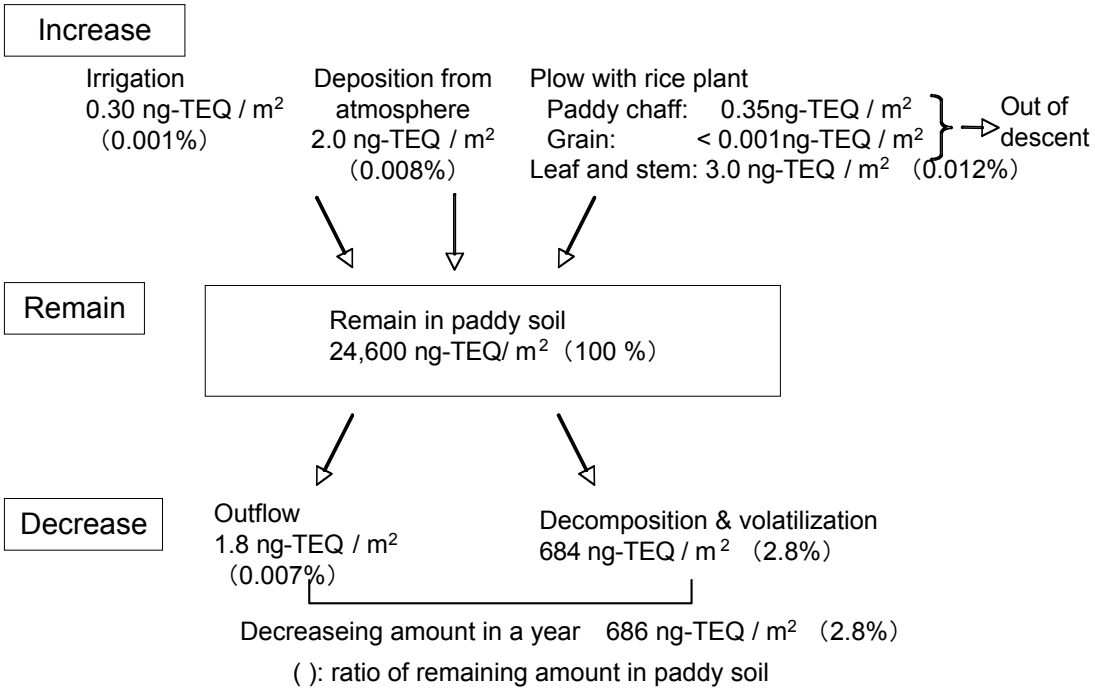

Fig. 2 Annual balance of dioxins in a Japanese paddy soil

Input from irrigation: The average concentration in irrigation water was 0.25 pg-TEQ/L (environmental assessment data from MOE). The dioxin input from irrigation to paddy soils was estimated to be 0.30 ng-TEQ/m^2.

Dioxin concentrations in agricultural chemicals, including fertilizers, were negligible. Therefore, the total increase in dioxin levels was calculated to be 5.3 ng-TEQ/m^2.

3. Factors that decrease dioxin levels

It is impossible to measure the decomposition and volatilization of dioxins in soils. Therefore, we tried to estimate this loss from the half-lives of these chemicals remaining in the soil. The mean amount of dioxins remaining in the NIAES paddy soils was 24 600 ng-TEQ/m^2. MAFF estimates the reduction half-term to be 25 years. From this estimate, we calculated a disappearance rate constant (K) equal to 0.0277 (/year). As a result, the annual decrease in dioxin levels in the NIAES paddy soil was calculated to be 681 ng-TEQ/m^2. This net decrease in dioxin concentration is the balance between the input and the output, including decomposition. The gross decrease is composed of runoff from the field, decomposition, and volatilization. Because our experimental data showed that the mean runoff of dioxins through drainage during flooding was 1.8 ng-TEQ/m^2, the decrease in dioxins due to decomposition and volatilization was 684 ng-TEQ/m^2.

4. Annual mass balance of dioxins in a Japanese paddy field

The results of these calculations are summarized in Figure 2, with the ratio of each amount to the total remaining in the soil shown in parentheses. Compared with the amount remaining in the soil, the amounts of increase and decrease in dioxins are very small, indicating that there has been little change, or only a very slow decrease, in dioxin concentrations in paddy soils. (R. Uegaki, N. Seike, and T. Otani)