Pesticides

Analytical chemistry – metrology in chemistry in particular – is currently undergoing major developments as a result of high demand from society for measurement of chemical compounds in the environment, health and agri-food sectors. In each of these fields, along with other industrial sectors, thousands of laboratories carry out millions of analyses every year. The metrological traceability of these measurements is not always adequately ensured in sensitive sectors such as environment and health. Pesticides are a prime example of a field where metrology in chemistry can play a significant role. The level of water pollution caused by these compounds is alarmingly high and numerous laboratories are analysing pesticides on a daily basis. Unfortunately, despite the implementation of sophisticated analysis techniques, traceability to the SI system is not always proved. This document covers the question of pesticides in general and reviews the analytical and metrological aspects.

Definition and history

Definition

In the texts relating to national and EU regulations, pesticides are also referred to as "plant protection products". European Directive 91/414/EEC of 15 July 1991 [1] concerning the placing of plant protection products on the market, defines them as:

active substances and preparations containing one or more active substances, presented in the form in which they are supplied to the user, intended to:

- *protect plants or plant products against all harmful organisms or prevent the action of such organisms*
- *influence the life processes of plants, other than as a nutrient (eg. growth regulators)*
- *preserve plant products, in so far as such substances or products are not subject to special Council or Commission provisions on preservatives*
- *destroy undesired plants, or*
- *destroy parts of plants, or check or prevent undesired growth of plants.*

Pesticides may be defined more simply as substances whose chemical properties contribute to the protection of cultivated and harvested crops. They may also be used to maintain non-agricultural areas such as public parks, transport infrastructures and private gardens.

The term "pesticide residues" describes the surplus substances present in the environment or in products following the use of a pesticide. This term covers both the compound and its degradation products.

Pesticides are formulations containing one or more chemical substances which may be inorganic or organic, synthetic or natural. Most pesticides used today are organic compounds, a small number of which are extracted or derived from plants. In general they are compounds of two types of substance:

- one or more active substances which give the product its desired effect
- one or more additives which increase the effectiveness, safety and ease of use of the product.

Pesticides may also be used to control plant growth and preserve crops. They can improve the quantity and quality of foodstuffs and reduce the spread of diseases that can be transmitted to man. They are nevertheless toxic products and as such present a potential danger for humans, animals and the environment.

History

Pesticides have been used in agriculture since antiquity. Their development since 1900 has gone hand in hand with advances in inorganic chemistry. The pesticides used in the early 20th century were derived from inorganic compounds or plants, such as compounds of arsenic, copper, zinc, manganese or nicotine sulphate. With the rapid development of organic chemistry in the 1940s, more sophisticated products appeared. Largely based on synthetic compounds, they resulted in the subsequent widespread use of pesticides (see figure 1).

Development of products			
	HERBICIDES	**FUNGICIDES**	**INSECTICIDES**
Before 1900	Copper sulphate Iron sulphate	Sulphur Copper salts	Nicotine
1900-1920	Sulphuric acid		Arsenic salts
1920-1940	Dinitro		
1940-1950	Phytohormones		Organochlorates Organophosphorates
1950-1960	Triazines Substituted ureas Carbamates	Dithiocarbamates Phthalimides	Carbamates
1960-1970	Dipyridyls Toluidines, etc.	Benzimidazoles	
1970-1980	Aminophosphonates Propionates, etc.	Triazoles Dicarboximides Amides, Phosphites Morholines	Pyrethroids Benzoylureas (growth regulators)
1980-1990	Sulfonylureas		
1990-2000		Phenylpyrroles Strobilurins	

Figure 1: Development of the three main types of pesticide from 1900 to today (Source: French Senate website)

Classification

The pesticides on the market today are characterized by a multiplicity of chemical structures, functions and activities, making classification difficult. In general, they may be classified in two ways, according to the type of parasite to be resisted or according to the chemical nature of their principal active substance.

Plant protection products are essential for agricultural production. There are a great variety, encompassing over 900 active substances and over 8 800 commercial products[1]. Moreover, the varieties and quantities used vary from country to country. The two classification systems are universal, however.

The first classification system is based on the **type of parasite** to be controlled. The three main families are herbicides, fungicides and insecticides.

Herbicides are the most widely used pesticides in the world. They are intended for elimination of plants competing with the crops to be protected by slowing their growth. Herbicides act on plants in a variety of ways. They can:

- disturb regulation of auxine (the main hormone acting on cell growth)
- inhibit photosynthesis
- inhibit cell division
- inhibit lipid synthesis
- inhibit cellulose synthesis
- inhibit amino acid synthesis.

Fungicides fight the spread of plant diseases caused by fungus or bacteria. Like herbicides, fungicides act on plants in different ways. They can:

- inhibit respiration
- inhibit cell division
- inhibit biosynthesis of amino acids or proteins
- disturb the metabolism of carbohydrates.

Insecticides are used to protect plants from insects by eliminating them or preventing them from reproducing. There are various types, including:

- neurotoxins
- growth regulators
- cell respiration inhibitors.

Apart from these three main pesticide families, other families include:

- acaricides (to eliminate mites and other acarids)
- nematicides (to eliminate the nematode group of worms)
- rodenticides (to eliminate rodents)
- mole killers (to eliminate moles)
- mollusc killers (to eliminate slugs and snails)
- corvicides and crow repellents (to eliminate crows and other crop-destroying birds).

[1] Source: French Crop Protection Industry Association (UIPP).

The second classification system is based on the **chemical nature** of the principal active substance. The main chemical groups are:

- organochlorine compounds
- organophosphates
- carbamates
- pyrethrinoids
- triazines
- substituted ureas.

As there are a wide range of pesticides on the market, there are a very large number of chemical families. It is difficult to classify certain pesticides as they may be used to eliminate two or more groups of parasites and therefore contain several active substances.

Market for plant protection products

Pesticides are among the substances liable to harm both human health and the environment. This problem is all the more pressing in France, as it is the world's third largest producer of plant protection products, behind the United States and Japan [2].

France is also Europe's leading consumer of pesticides, well ahead of Germany and Italy. Figure 2 below shows the distribution in Europe of the plant protection products market.

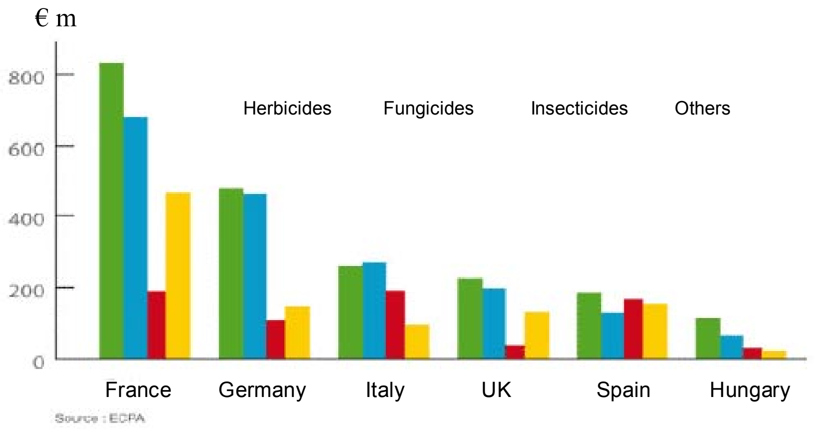

Figure 2: Consumption of the three main families of pesticides in Europe (Source: ECPA)

The leading position of France is explained by the fact that over half of the country's territory is agricultural land. Annual French consumption of active substances is around 100 000 tonnes. Although most products are used for agricultural purposes, non-agricultural uses represent up to 10% of total consumption.
Figure 3 below shows pesticide sales in France in recent years.

Figure 3: Sales of plant protection products in France from 1999 to 2006 (Source: UIPP)

Sales of plant protection products in France have declined since 1999. This phenomenon should be interpreted with caution, however. The downward trend may be due to increased awareness of the harmful side effects of pesticide use, but it may also be explained by the development of new active substances over the last few years. These substances are usually more toxic, hence more effective in low doses.

Pesticide contamination

Pesticides have been found in all environmental compartments – soil, river water, ground water, rainwater and air – for almost fifty years now. They have also been found in fruit, vegetables, cereals and products of animal origin.

Non-respect of Good Agricultural Practices can lead to contamination of the three biosphere compartments – soil, water and air. As pesticides may be found in all three compartments, their geochemical cycle is highly complex. Figure 4 below is a simplified diagram of the possible contamination when pesticides are used.

Pesticides are transferred to crops, food products or environmental compartments such as soil or water in several different ways:

- they are transferred to soil and growing crops during application
- they leach to ground water
- they drift from adjacent fields
- they are transported in waterways, rivers and lakes
- pesticide industry effluent is discharged into rivers and waterways, absorbed in soil and then transferred to crops [3].

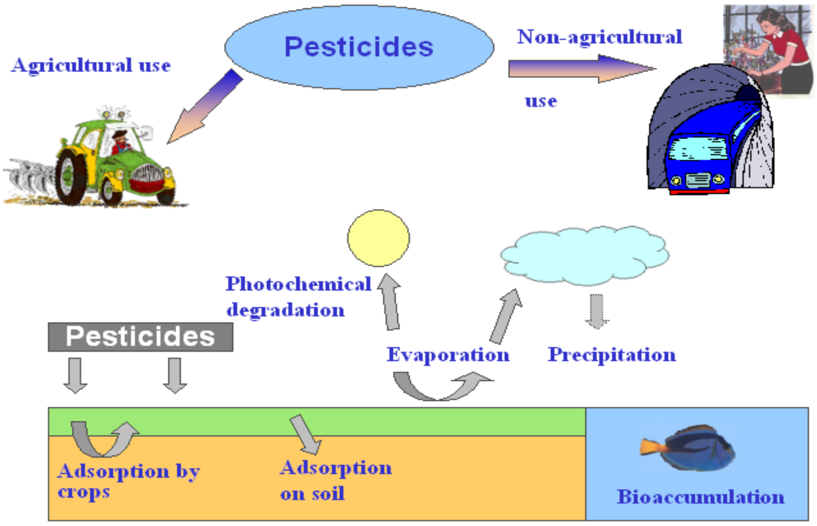

Figure 4: Some of behaviour of pesticides in the environment

It should be borne in mind that pesticides are not used only in an agricultural context. They are also used for non-agricultural purposes by local authorities, railway companies, industries, and private individuals maintaining gardens. Pesticide pollution is not therefore generated solely by agricultural activity.

In the agricultural sector, application of pesticides to crops may generate a range of phenomena. At present, however, pesticide dispersion is very poorly controlled. This is a complex problem because dispersion of pesticides in the environment depends not only on local soil and hydrological characteristics but also on climatic conditions. When crops are sprayed, between 10% and 70% of the product may be lost on the ground. Active substances may therefore be adsorbed by plants or the soil. Pesticides may also evaporate during spraying operations, with between 30% and 50% lost in the air.

After evaporation pesticides may accumulate in clouds, resulting in the presence of active substances in rainwater. Photochemical degradation of certain compounds may also occur. Leaching, polluted rainwater and drift during application may cause contamination of aqueous environments and result in bioaccumulation in aquatic life.

Effects on health and regulations

Effects on health

The use of pesticides brings undeniable advantages by increasing agricultural yield. However, pesticide residues may be found in foodstuffs and constitute a potential risk for consumers.

The large number of pesticides on the market present varying levels of toxicity for human beings. They range from compounds that are highly toxic to others that are practically harmless for mammals. Some products may present a high level of acute toxicity yet be eliminated easily by the organism, whereas other substances presenting lower acute toxicity may accumulate in the organism and result in longer-term effects. At the same time these products are converted into metabolites liable to have other repercussions on the human organism.

Pesticides are therefore known to have certain harmful effects on health. Human beings can absorb them in food and water, through contact with the skin, or through inhalation. The health risks due to exposure to pesticides may be linked to acute intoxication of users. Long-term risks are difficult to estimate, however. Various studies carried out around the world have focused on the impacts on health of pesticide use.

In scientific literature, it has been shown that pesticide residues cause reproduction or development problems and nervous system disorders [4].

Human health risks vary according to the type of pesticide and the amount of exposure. Moderate risks resulting from poorly controlled application of pesticides include headaches, skin rashes or eyesight problems [5]. A study carried has shown that exposure to pesticides could lead to weakening of the immune system [5], while the Meyer team has shown that prostate and stomach cancers occur more frequently among farmers [6]. Some pesticides are also considered to be endocrine disrupters, ie. they interfere with hormones by simulating their action. This can have a harmful effect on living beings.

Exposure to low doses could therefore have long-term health consequences for consumers. A study carried out in the United States has shown the presence of pesticide residues in different matrices: urine, blood, adipose tissue and breast milk (source: French government, Observatoire des Résidus de Pesticides). The presence of pesticides in breast milk could explain poor foetal development and congenital malformation [7]. Pesticides and their degradation products have also been identified as agents liable to affect male fertility, particularly via testicular toxicity [8].

Some pesticides are now recognized as persistent organic pollutants. Their main characteristics are:

- environmental persistence
- accumulation in fats
- dispersion in the environment via atmospheric and sea currents
- harmful effect on health.

Many of these pesticides are insecticides, such as endrin, aldrin, dieldrin, chlordecone, lindane and chlordane.

Regulations

Foods are complex mixtures of natural compounds such as lipids, carbohydrates, proteins and vitamins. They may also contain other substances originating from industrial processes, agrochemical treatments or packaging products. Although toxic compounds such as pesticides, toxins or polyaromatic hydrocarbons (PAHs) may be present in very low quantities, they can nevertheless be dangerous to health. The risk of toxicity has led the authorities to introduce strict regulations [9].

Pesticide levels are continually monitored and covered by regulations. Standards for authorized pesticide residue levels in water or foods of various origins are covered in particular by European Directive 91/414/EEC [1]. The main objective of the European Union's phytosanitary legislation is to ensure the safety of foodstuffs produced from plants by guaranteeing the health and quality of crops in all Member States. The approval procedures for active substances are inevitably complex.

Public health authorities have also introduced **maximum residue levels** (MRLs). Pesticide MRLs correspond to maximum expected quantities, established according to the Good Agricultural Practices (GAPs) set when authorization is obtained to place a pesticide on the market. MRLs reflect the use of the minimum quantities required to protect crops effectively, resulting in acceptable residue levels (ie. levels that have no effect on health). When pesticides are applied in accordance with GAPs, MRLs are not exceeded. Deviation from GAPs can lead to the presence of harmful residues, resulting in health risks [10].
The maximum residue levels fixed for an active substance and a given crop are based on toxicological, ecotoxicological, agronomic and biological studies.

MRLs are defined at national, European and international levels. European Directives take precedence over national law. The importance of European MRLs may cause a Member State to publish MRLs for an active substance contained in a product that is not approved within its territory. MRLs have been set for water, fruit and vegetables, and for each individual pesticide or group of pesticides. The industrial market for plant protection products is undergoing constant development, particularly concerning new molecules. Most newly developed molecules are effective in very small amounts, so authorized MRLs are correspondingly low. This means that estimating the molecules in the different products is a complex process.

It is therefore in this context – regulating the presence of pesticides, ensuring marketed products comply with regulations, and protecting consumers – that analysis methods able to identify pesticides accurately and quantify them at very low levels are urgently required.

Pesticide analysis

Analysis of pesticide residues in the various environments likely to have been polluted is a difficult process. Several different techniques must be used, for three main reasons:

- plant protection products belong to a very wide range of chemical classes
- they are applied on, or are likely to be found on, a wide variety of matrices (water, foodstuffs, soil, sediments, plants, etc.), with possible interferences
- detection limits are becoming lower and lower as safety levels rise.

Analysis methods aim to develop and validate tools to identify and quantify pesticides in the different compartments of the environment, where concentration levels can be very different. There is no system for analysing pesticides directly, so in the case of water there must be a trapping stage where compounds are adsorbed on a solid support. The pesticides are then extracted from the solid support and analysed. If pesticides are present in a solid matrix, they must be extracted with a solvent. In the case of liquids, the quantity adsorbed on the support is directly proportional to the concentration in the environment, and must be high enough to be detected by chromatography techniques[1].

A wide variety of molecules are likely to be retained on the support. Once the specific pesticide extraction procedure has been completed, the extract must therefore be purified in stages to eliminate compounds that may interfere in the actual analysis phase. Compounds must be identified with maximum certainty, so analysis usually takes the form of chromatography[1] combined with mass spectrométry[2].

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[1] Chromatography is a technique for separating the chemical compounds in a mixture that hinges on the difference in partition affinity among the components to be analysed between a mobile phase and a stationary phase (contained in a column). In gas chromatography the mobile phase is a gas. Separation of the compounds depends mainly on their specific affinity with the stationary phase. In liquid chromatography (eg. high performance liquid chromatography, HPLC) the mobile phase is a liquid, such as a water-methanol or water-acetonitrile mixture.

[2] Mass spectrometry is a technique used to determine the composition of a compound by breaking down a molecule under the impact of electrons or other molecules and separating the fragments (according to their mass) in a magnetic and/or electric field. Identification of the fragments makes it possible to identify the initial molecule. The mass spectrum of a molecule is comparable to a fingerprint.

Current analysis methods do not yet allow us to identify and quantify all pesticides on the market with one sole procedure. However, over the last ten years multi-residue methods have been developed that make it possible to measure almost 100 compounds with only one chromatographic injection.

In France, the analysis methods to be used for public health checks are specified (along with performance levels in some cases) in three government orders:

- The *arrêté* of 29 November 1991 (modified) specifies methods for analysing samples of water from swimming places.
- The *arrêté* of 17 September 2003 specifies methods for analysing samples of water intended for human consumption and samples of raw surface and ground water used to produce water for human consumption.
- The *arrêté* of 15 November 2004 specifies the performance characteristics of methods for analysing samples of conditioned natural mineral water.

Metrological analysis methods

In view of the large number of active substances and increasingly low MRLs, the analysis techniques used to analyse compounds must be reliable, highly selective and capable of detecting and quantifying at low content levels. The optimum tool for precise analysis of the physico-chemical composition of pesticides is liquid chromatography used in tandem with mass spectrométry (HPLC/MS²).

The analysis methods developed to monitor pesticide residues usually involve extraction of compounds from the matrix to be studied, followed if necessary by purification of the extracted compounds before they are identified and quantified. An entire analysis protocol must therefore be implemented and validated to ensure the analysis is controlled and reliable.

The measurements made by water analysis laboratories must be traceable to the SI system to guarantee their reliability and comparability. Laboratories can ensure traceability by using certified reference materials (CRMs) that enable them to validate their analysis protocols. These reference materials are generally produced by national metrology laboratories.

Certification of reference materials requires the use of a high-precision metrological method called the primary method.

Isotope dilution (ID) – simultaneous measurement of a molecule and its isotopically labelled homologue – is the technique generally used in metrology in chemistry. It is supported by analysis techniques such as liquid chromatography combined with mass spectrometry (ID/HPLC/MS). When isotope dilution is combined with a metrological protocol, relatively low measurement uncertainties can be obtained.

If a triple quadrupole analyser is used, total separation of all analysed compounds with identical molecular ions is not necessary. By observing the transition from molecular (parent) ions to fragment (daughter) ions, compounds can be detected fairly selectively.

Molecular ions and fragment ions can be detected by means of electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). Detection with ESI is more appropriate for most compounds studied, as APCI involves heating the source to high temperatures (350°C to 550°C), making it very difficult to detect thermolabile molecules.

Depending on the compound, the detection limits usually obtained vary from 0.5 to 10 pg/µl, while quantification limits vary from 1.5 to 30 pg/µl. These very low values confirm the efficacy of the liquid chromatography technique combined with mass spectrometry.

At international level, in 2005 eight national metrology laboratories completed an interlaboratory comparison to analyse chlorinated pesticides in solution. It was performed as a key comparison for the Consultative Committee for Amount of Substance – Metrology in Chemistry (CCQM) of the International Committee for Weights and Measures (CIPM).

This comparison (CCQM-K39) focused on determining four pesticides (4.4'-DDE, 4.4'-DDT, Lindane, trans-Nonachlor) in iso-octane, in the presence of four other pesticides. The concentrations of the four target pesticides ranged from 10 ng/g to 600 ng/g. The final report of the comparison has not yet been published, but results from the laboratories are in line with the gravimetric value to +/-5%, which is highly satisfactory. The CCQM plans to organize further international comparisons covering other pesticides and more complex matrices.

The number of pesticide CRMs available to water analysis laboratories is highly insufficient. Considering the importance of pesticide analysis, particularly for application of the EU Water Framework Directive (2000/60/EC), European national metrology laboratories have a significant role to play in ensuring the traceability of measurements.

LNE has been running a CRM research programme for several years in partnership with the Environment and Analytical Chemistry Laboratory (UMR CNRS 7121) of the Industrial Physics and Chemistry School (ESPCI) in Paris. The purpose of the programme is to produce matrix certified reference materials for distribution to analysis laboratories. These CRMs are used in the environment field (river water matrix) and the agri-food field (cereal matrix). The analysis methods developed apply to current pesticides recently placed on the market.

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[4] Report by French Ministry of Health and Social Affairs on pesticides in drinking water

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Journal of Agriculture and Food Chemistry, 2005, 53(3), pages 528-537

Annex II: Links

Links from www.observatoire-pesticides.gouv.fr

International and American organizations

- World Health Organization (WHO), pesticides:
  http://www.who.int/topics/pesticides/en/
- Food and Agriculture Organization of the United Nations (FAO):
  http://www.fao.org
- United States Environmental Protection Agency (EPA), pesticides:
  http://www.epa.gov/pesticides/
- Organization for Economic Cooperation and Development (OECD), pesticides:
  http://www.oecd.org/
- Organization for Economic Cooperation and Development (OECD), biocides:
  http://www.oecd.org/department/

European organizations

- European Commission, agriculture:
  http://europa.eu.int/comm/agriculture/index_en.htm
- European Commission, agriculture and food:
  http://europa.eu.int/comm/agriculture/foodqual/index_en.htm
- European Commission, food safety:
  http://europa.eu.int/comm/agriculture/foodqual/index_en.htm
- European Commission, plant protection:
  http://europa.eu.int/comm/food/plant/index_en.htm
- European Commission, evaluation and authorization of products:
  http://europa.eu.int/comm/food/plant/protection/evaluation/index_en.htm
- European Commission, biocides:
  http://europa.eu.int/comm/environment/biocides/index.htm
- European Commission, maximum residue levels:
  http://europa.eu.int/comm/food/plant/protection/pesticides/index_en.htm
- European Commission, sustainable development:
  http://europa.eu.int/comm/environment/ppps/home.htm
- European Food Safety Authority (EFSA)
  http://www.efsa.eu.int/index_en.html

French government

- Presidency:
  http://www.elysee.fr/
- Ministry of Agriculture and Fisheries:
  http://www.agriculture.gouv.fr
- Ministry of Ecology and Sustainable Development:
  http://www.ecologie.gouv.fr/
- Ministry of the Economy, Finance and Industry:
  http://www.minefi.gouv.fr
- Ministry of Health and Social Affairs:
  http://www.sante.gouv.fr/
- Prime Minister's Office:
  http://www.premier-ministre.gouv.fr/fr/

French agencies

- French Agency for Environmental and Occupational Health Safety (AFSSET): http://www.afsset.fr
- French Food Safety Agency (AFSSA): http://www.afssa.fr
- French Institute for the Environment (IFEN): http://www.ifen.fr
- National Institute for Public Health Surveillance (INVS): http://www.invs.sante.fr

French research bodies

- National Institute for Agricultural Research (INRA): http://www.inra.fr
- Agricultural and Environmental Engineering Research Institute (CEMAGREF): http://www.cemagref.fr
- National Scientific Research Centre (CNRS): http://www.cnrs.fr
- National Institute of Health and Medical Research (INSERM): http://www.inserm.fr
- National School of Public Health (ENSP): http://www.ensp.fr
- National Institute of the Industrial Environment and Risks (INERIS): http://www.ineris.fr
- French Research Institute for Exploitation of the Sea (IFREMER): http://www.ifremer.fr

Thematic portals (French government)

- Water (eaufrance): http://www.eaufrance.fr
- Health bodies: http://www.sante.fr/
- Health, environment, work (Santé Environnement Travail): http://www.sante-environnement-travail.fr

Professional associations

- European Crop Protection Association (ECPA): http://www.ecpa.be/website/index.asp
- French Crop Protection Industry Association (UIPP): http://www.uipp.org

Environmental bodies

- France Nature Environment (FNE): http://www.fne.asso.fr
- Movement for the Rights and Respect of Future Generations (MDRGF): http://www.mdrgf.org