Antibody based methods for environmental and food analysis: a review

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ABSTRACT: Antibodies have widely been used as analytical tools in various assays and techniques developed for clinical chemistry and endocrinology and for food and environmental research and risk control. Antibody development in the Veterinary Research Institute, Brno, and their application in ELISA and related techniques such as immunosensors has been directed especially to phenoxyacetic acid herbicides, s-triazine herbicides, sulfonylurea herbicides, polychlorinated biphenyls, surfactants (linear alkylbenzene sulphonates) and toxic metabolites (nonylphenol), and selected veterinary drugs (namely nitrofurans and sulfonamides). This paper provides an overview of progress achieved in the production of key immunoreagents in this laboratory (and in some cooperating laboratories) during the last 15 years. A comprehensive analysis of papers published on immunoassays and biosensors used in food and environmental research since 1980 demonstrates a rapid increase of publications on "ELISA and immunoassays" since 1991 (more than 500 papers were published each year since 1996). More than 200 papers on "biosensors" have been published each year since 2001. Atrazine was the most frequently found key word with ELISA and immunoassays: 438 papers were written by 971 authors from 308 institutions. The Web of Science® database is a useful tool for an assessment of the researcher's and institution's interest in the specific topics of research.

Keywords: ELISA; immunoassay; biosensor; food analysis; environmental research

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1. Introduction

Immunochemical methods, which have been used for decades in clinical chemistry as rapid, simple and reliable tools of screening analysis, have gradually spread to veterinary medicine, agriculture and other areas including environmental and food analysis. Antibodies "made to measure" are required for both single and simultaneous measurement of multiple analytes (Spinks, 2000). Chemical pesticides, polychlorinated biphenyls (PCBs) and antibiotics are typical examples of contaminants

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which can be rapidly and efficiently determined by antibody based methods. Antibodies enable not only a rapid detection of analyte in water, body fluids, soil or food extracts, but they can also be exploited in sample preparation prior to analyte detection. Because antibody affinity and specificity determine primarily the analytical capability of the immunochemical method, the properties of the antibodies represent an important innovative factor in developing an analytical system. Highly sensitive detection of toxic analytes can be performed by enzyme immunoassays, immunosensors and related techniques while immunoaffinity chromatography and flow injection immunoassay systems enable the concentration and clean up of the analytes in question.

Low-molecular weight compounds are called haptens in immunology. Preparation of antibodies against haptens such as pesticides and PCBs is based on covalent binding of the hapten to a carrier protein and immunisation of animals by the synthesised immunogens. The manner of chemical binding of the hapten to a protein determines the character of the antibody specificity. Various types of hapten derivatives conjugated to proteins have been used for antibody development. Polyclonal antibodies are produced by using traditional immunisation procedures, namely in rabbits, goats, sheep and pigs. A certain drawback of the method lies in the fact that it is not possible to produce identical antibody specificity even in two animals of the same species. On the other hand, wide spreading of monoclonal antibodies is limited by low predictability of the hybridoma technology result. It can be noted that polyclonal (rabbit) antibodies are the most widely used reagents in immunochemical analysis so far. However antibody engineering and production of recombinant antibodies is a very promising field both for research and application (Hock, 2002; Kramer and Hock, 2003).

Basic synthetic ways of preparation of the hapten derivatives (hapten design) have been explored primarily in steroids in the eighties of the last century (Franek, 1987). Hapten immunochemistry thus represents a consistent area for the development and preparation of conventional antibodies. As new impulses for further progress are largely depleted, further experimental strategies have been sought outside the classical immunochemistry area. In the last decade, molecular biology has generated fundamental changes in antibody production. Preparation of recombinant antibody fragments with novel binding properties was a primary goal of gene technologies. Their major asset lies in the possibility to focus mutagenesis to that part of gene which determines the structure and affinity of antibody binding site. Large phage libraries expressing antibody fragments on the surface of individual phage particles were used for preparation of recombinant antibodies. The systems enable separation of individual phage particles and subsequent selection of phage antibodies from a large number of expressed phage particles.

In the Veterinary Research Institute, Brno, a wide variety of immunogens, antibodies and enzyme conjugates have been developed and incorporated into immunoassays, and related techniques. The objective of this paper is to provide an overview of progress achieved in the production of key immunoreagents for the determination of topical environmental and food contaminants in this laboratory in the course of the last 15 years. Not least of the aims has been an attempt to exploit the produced antibodies in biosensor technologies as cost-effective alternatives for screening analysis.

2. Immunochemical strategies, reagents and applications developed in the Veterinary Research Institute, Brno

2.1 Phenoxyacetic acid herbicides

The effort was focused on 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) representing the group of broadly used phenoxyalkanoic acid herbicides which can potentially contaminate ground water supplies. 2,4-D and MCPA molecules were conjugated to protein via their carboxyl group by the conventional mixed anhydride chemistry, forming the desired herbicide-protein conjugates. The conjugates served as immunogens for the immunization of animals or as coating antigens in indirect ELISA, polarisation fluoroimmunoassay (Eremin et al., 1991) or immunosensors (Skladal et al., 1994). A panel of specific monoclonal antibodies against 2,4-D was produced in this laboratory by various clones (Franek et al., 1994). Indirect and direct assays employing these antibodies were optimized in respect of coating dilution, enzyme conjugate (tracer), pH, reagent volumes and incubation times to reach maximum assay sensitivity. The sensitivity of direct monoclonal ELISA was about 0.1 μg/l. The piezoelectric

immunosensor based on these monoclonal antibodies exhibited a limit of detection below 0.01 μg/l (Halamek et al., 2001). Monoclonal antibody (clone E2/G2) was used for construction of an affinity biosensor based on solid supported lipid membranes (Hianik et al., 1998). Additionally, scFv recombinant fragments against 2,4-D hapten were produced in this laboratory by naive phage library (Brichta et al., 2003). The antibody fragments showed a lower degree of specificity and stability but their practical utilisation as immunoassay reagents is limited at present. Identification of different hybridoma cell lines by DNA-sequencing that produce antibodies against herbicide 2, 4-D was reported in our recent work (Brichta and Franek, 2003). Interestingly, we were not able to produce specific antibodies against MCPA. The antibodies produced against MCPA-protein immunogens exhibited always high cross-reactivity with 2,4-D herbicide.

2.2 s-Triazine herbicides

Atrazine and simazine are the most common monochloro-substituted derivatives belonging to the s-triazine family. Owing to high persistence in the environment, accumulation of s-triazines in soil may cause ground water contamination. Moreover, atrazine carry-over may be harmful to certain rotation crops and therefore the soil should be analysed before planting. The hapten-protein immunogens were prepared by derivatizing atrazine and simazine and subsequent conjugation of the derivatives to proteins via aminohexane carboxylic and thiopropane carboxylic groups (Franek et al., 1995). The rabbit antibody produced allowed the determination of atrazine in water samples in the range of 0.02 to 5 μg/l in direct ELISA format. A polarisation fluoroimmunoassay for atrazine was optimised by structure changes of labelled antigen (Samsonova et al., 1994). Monoclonal antibodies raised against the same hapten-protein immunogens were produced in this laboratory and subsequently employed for development of piezoelectric (Pribyl et al., 2003) and amperometric (Killard et al., 2001) immunosensor. The monoclonal reagents incorporated into the immunoassay system were less sensitive compared with rabbit antibodies. ELISA methods using both rabbit and monoclonal antibodies were used for determination of atrazine in methanolic extracts of real field samples. Analysis of the soil samples by ELISA showed good correlation of results obtained by immunoassays and gas chromatography within the concentration range of 0–380 μg/kg (Deng et al., 1999). The data obtained by rabbit and monoclonal ELISA showed no difference although the sensitivity of the method using rabbit antibody was one order of magnitude higher than that based on monoclonal antibody. Additional monoclonal antibody (clone B10/B8/D2), having a generic pattern (broad cross-reactivity) for simazine and related s-triazines, was employed for the development of a highly sensitive flow through amperometric immunosensor based on the peroxidase chip and enzyme-channelling principle (Zeravik et al., 2003). Preparation of modified disposable screen-printed electrodes using enzyme-channelling can be a promising approach when the detection of extremely low analyte concentration is required. Monoclonal antibodies against atrazine, simazine and 2,4-D were used for the development of the flow injection immunoanalysis and compared with conventional immunoassays (Franek et al., 2000). ELISA was the more reliable technique in real screening analysis.

2.3 Sulfonylurea herbicides

The growing popularity of the sulfonylurea herbicides in the agriculture industry has necessitated analytical methods for water and soil matrices with good recovery and low detection limits. A single ring hapten strategy was suggested to produce generic antibodies with dominant selectivity towards arylsulfonyl or triazine moieties of metsulfuron-methyl herbicide. (Kolar et al., 2002). Superior monoclonal antibody (clone 2C8/C8) showed in indirect ELISA cross-reactivity among eight related sulfonylurea species and proved to be an effective generic reagent for this class of herbicides. The assay operated within the ppt-ppb calibration range. A decline in assay sensitivity was observed in monoclonal antibody batches originating from later production stages. It should be noted that such change in properties has not been observed in other monoclonal antibodies produced in this laboratory.

2.4 Polychlorinated biphenyls

PCB are recognized as ubiquitous environmental pollutants, because of their low rate of environ-

mental degradation and tendency to accumulate in tissues. They represent a class of 209 discrete congeners with 1–10 chlorine atoms attached to a biphenyl nucleus. In the Veterinary Research Institute, a considerable effort has been devoted to produce analytical antibodies against PCBs within the last 15 years. Goat antibodies, incorporated into radioimmunoassay, were applied to the screening of PCB in cow´s milk (Franek et al., 1992; Sisak et al., 1995). A correlation of results of immunoassay and gas chromatography (r = 0.67, n = 27) was found within the concentration range of 0.1–5.58 mg Aroclor 1260/kg milk fat (Sisak et al., 1995). Sheep antibodies raised against low-chlorinated PCB congeners (Aroclor 1242) were produced (Franek et al., 1997) and subsequently used for screening of PCB in soil extracts by ELISA (Deng et al., 2002). The antibodies were further used for a development of disposable electrochemical immunosensor for the detection of PCB in food samples (Laschi et al., 2000, 2003). Antibodies against highly toxic (coplanar) PCBs and respective enzyme tracers were developed in recent years (Franek, 2000; Franek et al., 2001a). The established direct ELISAs were highly sensitive (ppt-ppb level) and specific for non-ortho (coplanar) PCB 77, 126 and 169 congeners whereas assay response to more abundant but less toxic PCB congeners was very low or negligible. The antibodies in combination with alkaline phosphatase tracers are being employed at the University of Florence for development of electrochemical enzyme immunoassay for coplanar PCBs. Commercial ELISA kits for coplanar congeners were developed in collaboration with Abraxis LLC (USA).

2.5 Surfactants and nonylphenol

Due to the extreme amounts of surfactants emitted into sewage plants and the persistence of some of their known metabolites, such as nonylphenol, this is a group of environmental pollutants with the highest priority. Although legislators prescribe biodegradability of surfactants, these cannot be completely mineralized in biological waste treatment plants, thus they arrive together with their metabolites in rivers and some are found even in drinking water (Franek et al., 2001b). Rabbit antibodies against linear alkyl benzene sulfonates (LAS) have been produced and incorporated into direct ELISA format (Franek et al., 2001b) or polarisation fluoroimmunoassay format (Yakovleva et al., 2002). The direct ELISA detection limit for LAS (90% technical mixture of 4-dodecylbenzenesulfonic acid isomers) was about 2 µg/l. It appeared from the cross-reactivity results that the developed ELISA does not distinguish parent LAS homologues and isomers from their major carboxylic metabolites. Thus, measurement of real samples by the ELISA will provide screening values reflecting a total amount of LAS including their carboxylic derivatives. Polyclonal and monoclonal antibodies were prepared to establish direct assays for determination of nonylphenol and octylphenol (Zeravik et al., 2004). 4-nonylphenols are toxic metabolites on non-ionic synthetic detergens having oestrogenic effects and resistance towards biodegradation. Rabbit antibodies were employed for establishment of assays with sensitivity about 3 µg/l. Specificity of monoclonal antibody (clone 4H6) allowed a sensitive detection of the long-chain forms of 4-n-alkylphenols. No cross-reactivity interference was indicated for linear alkylbenzensulfonates and phenolic compounds. These antibodies were employed for development of ELISA and dipstick tests to measure nonylphenol in water samples (Samsonova et al., 2003) and for development of polarisation fluoroimmunoassay (Yakovleva et al., 2003). A rapid Biacore biosensor immunoassay of 4-nonylphenol was optimised and applied for shellfish analysis (Samsonova et al., 2004).

2.6 Veterinary drugs

The nitrofuran antibiotics were banned from use in food animal production in 1993–1995 in Europe and the Czech Republic because of concerns about their carcinogenicity and mutagenicity. Effective control of nitrofuran drugs (furazolidone, nitrofurantoin, furaltadone, nitrofurazone) can be achieved by measuring tissue concentrations of bound nitrofuran residues (3-amino-2-oxazolidinone (AOZ), 1-amino-hydantoin (AHD), 3-amino-5-methylmorpholino-2-oxazolidinone (AMOZ) and semicarbazide (SEM)) which are toxic metabolites of their parent compounds. Within the European project FoodBRAND, monoclonal antibodies for detection of AOZ, the metabolite of furazolidone, were produced and characterised in terms of specificity and sensitivity (Vass et al., unpublished yet). The superior antibodies, produced by clones 3B8/2B9 and 2D11/A4, were exploited for development of the simplified ELISA method

without using extraction and clean up operations (Diblikova et al., 2004 submitted). Detection capability of the method was 0.4 μg/kg for shrimp, poultry, beef and pork meat. An excellent correlation of results obtained by the ELISA and LC/MS-MS within the concentration range 0–32 μg/kg was found in the naturally contaminated shrimp samples and in the incurred poultry samples. A testing ELISA kit for AOZ was developed in collaboration with the Queen's University of Belfast and R-Biopharm, Darmstadt. An effort of this laboratory has been devoted to producing antibodies against sulfonamides. A highly sensitive immunoassay for determination of sulfadimidine in tissues was established (Franek et al., 1999). However, a need to detect various sulfonamide species within a single assay prompted us to develop antibodies for a broad-specificity test. At present, a panel of class-specific (generic) antibodies have been produced. Polyclonal antibodies with a broad specificity for simultaneous detection of 18 sulfonamides at the maximum residue level have been prepared and characterised in both direct and indirect ELISA formats (unpublished results).

3. Immunoassays in food and environmental research: Analysis of publications (1980–2004)

The trends in research can be followed by analysis of the published papers related to the specific topic used as a key word for searching in a suitable database[1]. Web of Science® database (Thomson-ISI, Philadelphia) offers not only the widest selection of journals indexed but the software tool for such analysis as well (Hruska, 2004). Immunoassays, namely in radioimmunoassay format (RIA), opened the new era in endocrinology in the seventies of the last century. In a certain sense, ELISA and biosensors seem to have assumed a similar importance in environmental and food science since the nineties. The increase of applications of ELISA is obvious from an index, ELISA/RIA, which is 0.65 and 1.87 in 1980–1990 and 1991–2004, respectively. The increase in the number of publications on RIA, ELISA and biosensors is 4.2, 12.1 and 28.3 times, respectively, in the same periods 1980–1990 and 1991–2004 (Table 1). These numbers of publications are clear evidence of the general importance of antibody based methods in recent decades.

Table 1. Number of publications
Source: Web of Science® database (Thomson-ISI, Philadelphia)

	1980–1990	1991–2004	Index
Radioimmunoassays	5 860	24 639	4.2
ELISA	3 821	46 152	12.1
Biosensors	420	11 871	28.3

The antibody based methods' significance for environmental and food analysis is evident from the data resulting from the analysis of published papers. In the nineties the number of publications dealing with ELISA or immunoassays and biosensors in food and environmental analysis dramatically increased (Figure 1). Only a few publications appeared until 1990, however since then the number of publications has increased to more than 500 and 200 per year on ELISA and biosensors, respectively. The numbers of publications, authors, institutions and journals publishing papers which appeared as search results using the keywords "ELISA or Immunoassays" or "Biosensors" and "Food", "Feed", "Water", "Soil" and "Air" are presented in Table 2. It is evident that 1 551 papers were published by 5 458 authors from 1 500 institutions in 509 journals on ELISA or immunoassays and food in 1980 to 2004. Feed, water, soil and air were also the subject of high number of publications both in association with ELISA or immunoassays and biosensors.

The analysis of search results for publications dealing with ELISA or immunoassays and different food and environmental contaminants are summarized in Table 3 and those on biosensors in Table 4. The Web of Science® database enables the researcher to find the authors and institutions most frequently publishing papers on a selected search profile.

Based on the criteria of a minimum of 30 papers published on each topic and 10% of these papers from one laboratory, the following institutions had

[1]It has to be noted that search results depend on the key words used: e.g. biosensor* is not able to distinguish immunosensors from other types of biosensors. Similarly the record listed for "ELISA AND ATRAZINE" could be selected due to the determination of IgG by ELISA after atrazine contamination.

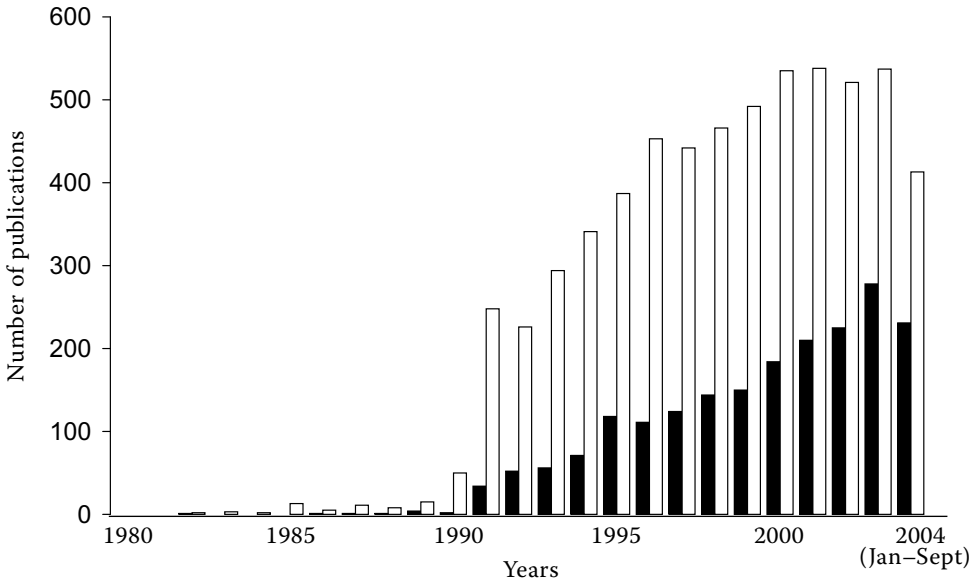

Figure 1. ELISA or immunoassay* (white bars) and biosensors (black bars) in food and environmental analysis
*used in the keyword for searching

the most productive teams in terms of publications on the selected topics:

ELISA or immunoassay (Table 3)

dioxin: University of California, Davis (16.2%)
nonylphenol: University of Bergen (13.5%), Moscow Lomonosov State University, Odense University and Veterinary Research Institute, Brno (10.8%)
sulfonylurea: Technical University of Munich (10.5%)
triazine or simazine: Technical University of Munich (10.3%)
zearalenone: University of Munich (20.6%), Michigan State University (11.8%)

Biosensors (Table 4)

triazine or simazine: US Naval Research Laboratory (16.4%)

Table 2. Number of authors, institutions and journals participating on publications
Source: database Web of Science® (Thomson-ISI Philadelphia), timespan: 1980–2004 (September)

	ELISA or immunoassays* and				
	Food	Feed*	Water	Soil	Air
Publications	1 551	1 004	2 305 #	674	468
Authors	5 158	3 930	6 398	1 823	2017
Institutions	1 500	1 099	1 771	572	625
Journals	509	473	696	218	244
	Biosensor* and				
	Food	Feed*	Water	Soil	Air
Publications	399	94	1 115	157	233
Authors	977	319	3 083	526	772
Institutions	361	122	886	185	275
Journals	155	63	299	72	112

*used in the keyword for searching
only 2000 records of the total 2305 were analysed (Web of Science analysis limit)

Table 3. Analysis of publications on ELISA or immunoassays
Source: database Web of Science® (Thomson-ISI Philadelphia), timespan: 1980–2004 (September)

	ELISA or immunoassay* and				
	Aflatoxin*	Antibiotic*	Atrazine	Dioxin*	Endocrine disruptor*
Publications	272	840	438	99	33
Authors	759	3363	971	312	145
AU 1	Chu FS 16	Martlbauer E 8	Hock B 41	Hammock BD 10	Hock B 3
AU 2	Santella RM 12	Stanker LH 8	Barcelo D 27	Gee SJ 8	(15) 2
AU 3	Groopman JD 9	Usleber E 8	Hammock BD 27	(3) 6	
Institutions	255	1012	308	144	55
INST 1	USDA ARS 20	John Hopkins Univ 14	Tech Univ Munich 42	Univ Calif Davis 16	Univ Bergen 3
INST 2	Univ Wisconsin 18	Univ Munich 14	CSIC 28	Univ Bergen 6	(7) 2
INST 3	Columbia Univ 12	Harvard Univ 11	Univ Calif Davis 28	CSIC 5	
	Furazolidone	Nitrofuran*	Nonylphenol	PCB #	Phenoxyacetic
Publications	4	1	37	133	8
Authors	9	4	156	414	30
AU 1	(9) 1	Caddell A 1	Arukwe A 4	Goksoyr A 10	Hammock BD 4
AU 2		Cooper KM 1	Franek M 4	Franek M 9	(5) 2
AU 3		Elliot CT 1	Goksoyr A 4	Mascini M 6	
		Kennedy DG 1	Korsgaard B 4	Van Emon JM 6	
Institutions	5	2	56	168	7
INST 1	(5) 1	Queens Univ Belfast 1	Univ Bergen 5	US EPA 12	Univ Calif Davis 4
INST 2		Vet Sci Div 1	Moscow Lomonosov St Univ 4	Univ Bergen 10	Hung Acad Sci 2
INST 3			Odense Univ 4	Vet Res Inst 9	(5) 1
			Vet Res Inst 4		
	Sulfonamid*	Sulfonylurea	Surfactant*	Triazine or Simazine	Zearalenone
Publications	87	38	239	349	68
Authors	263	128	871	739	188
AU 1	Sporns P 7	Knopp D 4	Kuroki Y 21	Hammock BD 33	Martlbauer E 14
AU 2	Haasnoot W 6	Niessner R 4	Akino T 18	Niessner R 21	Usleber E 13
AU 3	Usleber E 6	(5) 3	Takahashi H 12	Hock B 20	Pestka JJ 6
Institutions	105	52	269	246	74
INST 1	Univ Alberta 7	Tech Univ Munich 4	Sapporo Med Coll 13	Tech Univ Munich 36	Univ Munich 14
INST 2	Univ Munich 7	(4) 3	Sapporo Med Univ 9	Univ Calif Davis 33	Michigan State Univ 8
INST 3	ARS 4		Univ Giessen 7	CSIC 19	USDA ARS 6
	State Inst Qual Control 4				

*used in the keyword for searching; # PCB or (polychlorinated biphenyl)
Three or four authors and institutions having the highest number of publications are listed. Abbreviations as in the Web of Science
Numerals in parenthesis: number of authors or institutions with the same number of publications

Table 4. Analysis of publications on biosensors
Source: database Web of Science® (Thomson-ISI Philadelphia), timespan: 1980–2004 (September)

	Biosensor* and				
	Aflatoxin*	Antibiotic*	Atrazine	Dioxin*	Endocrine disruptor*
Publications	22	86	96	8	12
Authors	57	313	283	44	41
AU 1	Nikolelis DP 6	Sternesjo A 6	Nikolelis DP 7	(44) 1	Hock B 2
AU 2	Krull UJ 3	Gaudin V 4	Skladal P 6		Kim BW 2
AU 3	Maragos CM 3	Gustavsson E 4	Gauglitz G 5		Seifert M 2
	Mascini M 3		Hock B 5		
Institutions	22	109	112	17	17
INST 1	Univ Athens 6	Swedish Univ Agr Sci 6	Univ Athens 7	Kyushu Univ 2	Sungkyunwan Univ 2
INST 2	Univ Toronto 3	Biacore AB 4	Masaryk Univ 6	(16) 1	(16) 1
INST 3	USDA ARS 3	Univ Cambridge 4	(4) 4		
	Furazolidone	Nitrofuran*	Nonylphenol	PCB #	Phenoxyacetic
Publications	1	1	7	25	0
Authors	3	3	30	70	
AU 1	AbuZuhri A 1	Merino M 1	(5) 2	Mascini M 4	
AU 2	Diab N 1	Nunez-Vergara LJ 1		Barcelo D 3	
AU 3	Schuhmann W 1	Squella JA 1		Marco MP 3	
Institutions	2	1	11	26	
INST 1	Arab Amer Univ 1	Univ Chile	Ariake Natl Coll Technol 2	Univ Florence 4	
INST 2	Ruhr Univ Bochum 1		Nagasaki Univ 2	CSIC 3	
INST 3			Prefectural Univ Kumamoto 2	Univ Calif Berkeley 3	
	Sulfonamid*	Sulfonylurea	Surfactant*	Triazine or Simazine	Zearalenone
Publications	29	3	125	67	2
Authors	97	13	383	202	12
AU 1	Fierke CA 7	Marty JL 2	Vadgama P 8	Kusterbeck AW 8	(12) 1
AU 2	Thompson RB 7	Ortega F 2	Hu NF 7	Charles PT 5	
AU 3	Maliwal BP 5	(11) 1	Reshetilov AN 7	(8) 4	
Institutions	34	4	122	80	2
INST 1	Univ Maryland 7	Univ Perpignan 2	Russian Acad Sci 9	USN 11	Dublin City Univ 1
INST 2	Duke Univ 5	Russian Acad Sci 1	Univ Manchester 9	Geocenters Inc 6	Xavier Univ 1
INST 3	(3) 3	Soka Univ 1	Nanjing Univ 8	Lund Univ 4	
		Univ Newcastle Upon Tyne 1		Univ Athens 4	

*used in the keyword for searching; # PCB or (polychlorinated biphenyl)
Three or four authors and institutions having the highest number of publications are listed. Abbreviations as in the Web of Science
Numerals in parenthesis: number of authors or institutions with the same number of publications

4. Conclusions

The progress of development of new immunoassays and related immunotechnologies is still limited by the availability of antibodies with the desired affinities and specificities for given applications. Efforts are still to be made for developing antibodies for both common and new contaminants and pollutants. The current trend is for development of antibodies that enable detection of several targets within a single test based on a broad specificity antibody. To achieve this aim innovative strategies based on immunochemical and recombinant concepts should be designed to develop and produce such generic antibodies. Continued academic and industrial research offers additional less traditional methodologies (imprinted techniques, natural and artificial receptors, aptamers) for production of binding species against pesticides, dioxins, antibiotics and other compounds of interest. Considering these novel approaches, there is no doubt that classical antibodies will play an important role even in the future since they exhibit a high potential for sensitivity and desired selectivity. Various antibodies against pesticides and other chemical pollutants and contaminants are available today and these can be employed for research and development activities. However, there is no security that these antibodies (if not monoclonal) will be accessible from the commercial source in the same quality and quantity in the long-term period. Thus, projects depending widely on antibody reagents should rather rely on their own immunoreagent sources to facilitate the application of their technologies if they gain acceptance by users.

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