Anexo 2: Resumen en inglés

Food surveillance is an approach to obtaining an overview of areas of potential risk of consumer exposure to chemicals from the diet. The risk is assessed by comparing exposure to toxicological limits and this enables comparisons to be made between exposure of one population group and another. Importantly for Government Agencies with limited resources a well co-ordinated and systematic approach to food surveillance facilitates sensible prioritisation of risk assessment. Surveillance enables a pro-active approach to assessing exposure to food contaminants and the 'intelligence' for Government to make a measured response to possible food scares. Recognising these benefits the Basque Government initiated a food surveillance programme in 1990 using the total diet approach to assess exposure to recognised contaminants. This general approach was reinforced with monitoring of a number of target contaminants in individual foods.

The difficulties in producing meaningful data for trace level analysis of additives and contaminants in foods should not be underestimated. Work at an international level, under such programmes as the WHO GEMS-Food AQA scheme, have demonstrated that a significant proportion of even experienced food analysis laboratories produce unacceptable and inaccurate data on occasions. Mindful of these problems, in setting up this surveillance programme particular attention was paid to the use of certified reference materials, where available, to provide an external benchmark for analytical results. In addition, throughout the course of the work reported here, the laboratory took part in proficiency testing exercises (FAPAS) and demonstrated satisfactory performance for analysis of the analytes in question. This quality assurance aspect of the results reported here is critical, and means that the data can reliably be compared from year-to-year, and can reliably be used within the EU and internationally for comparative purposes.

This report provides for the first time a reliable and comprehensive overview of consumer exposure to chemical contaminants in the Basque Country over a six year period. The results provide information on trends in contaminants against which any future measurements can be assessed. The work reported here has enabled some priority areas to be identified and lays an important foundation for continued future food surveillance.

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The Basque Country is a small region in the north of Spain of 7,261 km² in area and a population of 2.2 million.

In 1990, the Basque Government initiated a Total Diet Study. He pioneered these type of studies in Spain. The study was designed on the basis of the experience of other countries which had been carrying out total diet studies for many years and it was decided that the market basket approach was most appropriate with the resources available.

The Total Diet Study is an important part of the Basque Government monitoring programme for chemical contaminants in the food supply. The primary purpose of this study is to provide estimates of the average intake of constituents of concern. It provides a picture of the norm and useful reference data, it shows trends in intakes and, occasionally, gives information about unexpected sources of food contamination checking the effectiveness of regulations and initiatives relative to levels of chemicals in foods. In addition, the results serve to guide other monitoring programmes. Apart from the estimation of the dietary intake of chemical substances which is a basic aspect of the food chemical surveillance, "selective controls" to determine residues of a particular contaminant in a particular food item have also been conducted. Also, for its particular characteristics it is worth mentioning here the Plan of Veterinary Drug Residues in Meat.

The design of the Basque Total Diet Study has been described in detail previously\(^6\). The types and quantities of foods that make up the average Basque 'Total Diet' are based on the results of a Food Survey carried out between 1988 and 1990\(^7\). This survey based on a representative sample of the adult population (25-60 yr) employed a "24h recall" interview and an individual food frequency questionnaire (n=2348). Information was collected on meals consumed outside and inside the home and on alcoholic and non-alcoholic beverages.

The main features of the study (Figure 2) can be summarised as follows: using the information provided by the Food Survey, the average diet of the population was established. Then the food list was prepared and the food items included (91) (Table 1) were purchased at monthly intervals in different locations of the Basque Country. After preparation and cooking, the foods were combined in groups (16 food groups) and analysed for the substances of interest. Finally, the intakes were calculated by a combination of these data with those of consumption, and compared with appropriate reference values and also with data from other countries. Caution, was exercised when comparing the intakes reported in different studies since in many cases the studies are not strictly comparable. If meaningful comparisons are to be made, published papers should include information on a number of points: the limit of determination of the analytical methodology, the assumptions made in intake calculations if concentrations are below the limit of determination, the weight and energy content of the diet and the manner of preparation of the diet. Also, the method used for estimating dietary intake has to be taken into account. In this document, we have compared our results with those obtained in other countries using the
market basket approach. Nevertheless, some comparisons with data from countries using other approaches have occasionally been made.

At present the prioritisation in the area of food chemical surveillance has been undertaken by a Working Group created by the Public Health Direction of the Health Department of the Basque Government. The Group was made up of 9 people devoted to different activities related to food control or food surveillance and included the Chairmen of the various Sub-Working Groups as well as a representative from the Laboratory. Its main task was to give an overview of the possible health risks in relation to food chemical substances and to establish priorities in that area. Also, it created draft-working documents, promoted exchange of opinions between participants in a certain field, designed definite programs, controlled their execution, interpreted the results, conducted evaluations and promoted new activities in each area. The Group has met regularly at monthly intervals and minutes of the meetings were sent to all interested parties. Up to now the 4 Sub-Working Groups have covered the following areas:

1. Sub-Working Group on Contaminants and Nutrients
   Heavy metals (mercury, lead, cadmium and arsenic), polychlorinated dibenzodioxins and dibenzofurans and polychlorinated biphenyls, mycotoxins (aflatoxin M1, aflatoxins B1, B2, G1 and G2), radionuclides and nutrients (iron, zinc and selenium)

2. Sub-Working Group on Pesticide Residues
   Organochlorine, organophosphorus, dithiocarbamates and N-methyl carbamates

3. Sub-Working Group on Additives
   Nitrate and nitrite, sulphite and boric acid

4. Sub-Working Group on Veterinary Drug Residues
   Thyrostatic agents, anabolic agents, antibiotics, sulfonamides and ß-agonists.

Some results of the Food Chemical Surveillance Programme have already been published. However, this report covers for the first time all the activities which have been carried out within the different surveillance areas in the period 1990-1995.

Heavy metals and arsenic

Lead and cadmium have been determined in all food groups since 1990. In 1990/91 they were determined in every total diet sample collected (every month) but since 1992 they have been
analysed in 6 total diet samples per year. Mercury and arsenic were also determined in all food groups in 1990/91 but after this period, as measurable amounts had only been detected in the fish group (and arsenic at very low levels in the alcoholic beverages group), they were only analysed in the fish group but every month (12 samples a year). Dietary intakes of these elements have been calculated assuming values below the limit of determination as being equal to the limit (Table 2).

**Lead**

The lead dietary intakes estimated in the period 1992-1995 varied from 26 to 33 µg/day with an average of 28 µg/day which accounts for 12% of the PTWI. These intakes are lower than those estimated in 1990-91 which were 46 and 40 µg/day respectively (Table 2) and therefore, from now on the discussion will refer to the value obtained by averaging the intakes of the last four years. It is important to note that for the estimation of lead intake, the possible contribution of drinking water has not been considered. The reason for this was that the limit of determination of the analytical technique was high (10 µg/L) and although the majority of the values were below that limit, if the most conservative approach was used (values below the limit equal to the limit), 10 µg more should have to be added to the total lead intake for an average consumption of 1 litre per day (this means nearly an extra 40% of the calculated intake). Subsequently, the limit of determination of the analytical technique has been lowered and this will now enable us to include the contribution of drinking water to the estimated lead intake in the future.

There are little differences in lead intakes reported from different countries. Lead intake in the Basque Country is higher than that reported for Sweden (17 µg/day), Finland (20 µg/day), USA (7 µg/day) and the Czech Republic (20 µg/day) and very similar to that of the UK (28 µg/day), Germany (29 µg/day), The Netherlands (29 µg/day) and Canada (28 µg/day) (Figure 3).

As regards to the sources of lead, this element is present in small amounts in most of the food groups of the diet and in this study alcoholic beverages, bread, fruits, fish and vegetables have been identified as major contributors in descending order (Figure 4). It is striking to note that alcoholic beverages contribute significantly to the total lead intake. This could be attributed to the high consumption of alcoholic beverages (243 g/day) and particularly to the high wine consumption (174 g/day). Lead levels in wines are considerably higher than those found in other beverages although lead concentrations in Spanish wines are similar to those found in other countries. However, it has to be considered that alcoholic beverages are not included as a group in the Total Diet Studies of some countries and therefore their contribution to the total lead intake is not always considered.
The average dietary cadmium intake in the period 1990-1995 is 11 µg/day and it accounts for 16% of the PTWI (Table 2). The intake of this element has remained fairly constant for the last 6 years (Figure 5). Cadmium intake in the Basque Country is among the lowest reported in the literature (Figure 6) and is in good agreement with those reported for Germany (11 µg/day), Finland (10 µg/day), Sweden (12 µg/day) and the USA (12 µg/day).

In this study, vegetables as a whole (potatoes + vegetables) and particularly potatoes are the highest contributors to the cadmium intake. Also fish, bread and to a lesser extent meat (Figure 7) contribute significantly to total cadmium intake.

- Lead is widely spread in small amounts in most foodstuffs of the diet and the food groups which contribute most to the total lead intake are alcoholic beverages, bread, fruits, fish and vegetables.
- The average lead intake is 28 µg/day which accounts for 12% of the PTWI and it is similar to that estimated in other countries.
- The coming EU regulations and the effects of the previous lead in petrol limitation, makes it foreseen the downward trend in lead intake observed in the last few years.

• The average dietary cadmium intake is one of the lowest reported in the literature (11 µg/day) and it accounts for 16% of the PTWI.
• The main contributors to the total cadmium intake are vegetables (particularly potatoes), fish and bread.
The dietary intake of mercury from the fish group varies between 10 and 13 µg/day (Figure 8). However, if values below the limit of determination are taken as the limit, the average intake in the period 1990–1995 would be 18 µg/day which accounts for 37% of the PTWI of 5 µg/kg body weight/day\(^1\).

Although speciation has not been conducted, most published analytical reports generally confirm that methylmercury, the most toxic form, accounts for around 75% of the total mercury content in the edible fillet of fish. Assuming this percentage, and taking into account that most species included in the total diet study are of marine origin, the average intake of methylmercury through the diet should be approximately 9 µg/day which accounts for the 28% of the PTWI of 3.3 µg/kg body weight for this compound\(^1\). This level of intake appears not to pose any health risk particularly considering that the predatory species such as ocean tuna, shark and swordfish which may contain highest methylmercury concentrations are consumed at very low levels\(^6\).

The estimated mercury intake in the Basque Country is high in relation to other countries (Figure 9). Our intake is much higher than that of countries like the UK (2 µg/day), The Netherlands (0.7 µg/day) or The Czech Republic (0.7 µg/day) where fish consumption is much lower. It is important however to note here that the treatment of values below the limit of determination is not the same in all the studies included in Figure 9. However, even considering only the intake of mercury from the fish group, mercury intake in the Basque Country (12 µg/day) is greater than any other reported.

Mercury concentrations in the fish group are not particularly higher than those from other countries and the high mercury intake is attributed mainly to a very high fish consumption. However, as fish species are analysed in an single food group, comparisons with data from other studies have to be made with caution because different fish groups of the various studies include different species.

In order to determine which fish species had the highest mercury levels, individual items included in the fish group of the 12 diets collected in 1995 were analysed separately. For this purpose, the analysis was conducted on uncooked fish samples and results compared with the limits established in the regulations\(^4\). The fish group includes 13 categories of foods, eight of which are specifically mentioned in all the diets whereas the remaining five represent a group of similar foods which share a certain characteristic (i.e. ‘other blue fish’ or ‘crustaceans’) (Table 1).

The average mercury concentrations of the fish species which are included in all the diets (12 samples in 1995) are low, (between 25 and 150 µg/kg) (Figure 10). As regards the mercury
content of other species, crustaceans always gave very low concentrations (less than 125 µg/kg) whereas high levels were found in a sample of sea bream (515 µg/kg) and in 2 samples of red mullet (485 and 1004 µg/kg respectively). Tuna fish which naturally accumulates higher concentrations of mercury, showed levels between 221 and 542 µg/kg although, in this species up to 1000 µg/kg is permitted.

Finally, a theoretical estimation of the mercury intake by extreme fish consumers has also been carried out. Several studies indicate that, as a rough rule-of-thumb, extreme consumers of food in general are unlikely to have an intake of food that exceeds twice the average consumption of the population as a whole. If a contaminant is mostly confined to an individual foodstuff within the diet, then ‘extreme’ consumers of that foodstuff within the population are unlikely to consume more than three times the amount consumed on average by the population’. Calculated mercury and methylmercury intakes according to this criteria by extreme consumers show that mercury and methyl mercury would account for 86-84% respectively of the appropriate reference values. However, the Basque Food Survey places the 95th percentile of consumption of fish at 355 g/day, which means that 5% of the population between 25 and 60 years consumes fish at or above this figure. This appears to indicate that mercury and methylmercury intakes could be well above the PTWIs for a certain range of the population. At present, a project to evaluate risks of exposure to mercury by extreme fish consumers is being prepared. This will include the estimation of real intakes as well as of the status of mercury in this group of the population.

- Mercury intake is the highest reported in similar studies in other countries and it accounts for 37% of the PTWI.
- Fish is the only source of mercury in the Basque Country and high mercury intakes are attributed to very high fish consumption (89 g/day).
- It is necessary to assess the exposure to mercury of risk groups of the population such as extreme fish consumers.

**Arsenic**

Total arsenic intakes estimated in most countries are usually very low (Figure 11) and when compared directly to reference values for inorganic arsenic it is concluded that there is no risk associated with the intake of this element.
even in the hypothetical case that all the arsenic present were inorganic. This argument thus avoids the necessity of conducting the determination of inorganic arsenic which is more tedious. However, in the case of the Basque Country, this approximation cannot be made because total arsenic intakes are very high and, if all the arsenic present were inorganic arsenic it would exceed the tolerable intake.

In 1990-1995 the average intake of total arsenic was 297 µg/day (between 255 and 345 µg/day) and it was higher than estimated in other countries including Japan (280 µg/day). Here again, high intakes are attributed to differences in fish consumption because most of the arsenic comes from this food group.

Although generally organic arsenic compounds which occur naturally at high levels in fish, shellfish and crustaceans, are considered less toxic than inorganic arsenic, due to high arsenic intake estimation, it was decided to carry out speciation of arsenic in the fish group of 12 total diet samples collected between 1990-1993. Results indicated that 84-100 % of the arsenic was present as arsenobetain and the maximum inorganic arsenic detected was 5% of total arsenic. This corresponds to a maximum inorganic arsenic intake of 15 µg/day which would account for 10% of the PTWI for inorganic arsenic (15 µg/kg weight)².

In order to estimate theoretically the possible health risk of extreme fish consumers, several assumptions similar to those considered for mercury, have been conducted. In this case, theoretical inorganic arsenic intake for the 95th percentile of the population would be below 40% of the appropriate PTWI.
Pesticide residues

The estimation of the dietary intake of pesticide residues started in 1990 with the surveillance of 16 organochlorine pesticide residues. In 1992, 17 N-methylcarbamates and dithiocarbamates were included, and in 1994 organophosphorus pesticide residues were also analysed. Dietary intakes have only been estimated for those residues that appear more than once above the limit of determination, and they have been estimated assuming values below the limit of determination as being equal to zero.

Organochlorine pesticide residues

16 organochlorine pesticide residues have been analysed in the 16 food groups of the diet (α, β, γ, and δ-HCH, HCB, heptachlor, heptachlor-epoxide, aldrin, dieldrin, endrin, α and β-endosulfan, DDT, DDD, DDE and methoxychlor) In 1990/91 10 total diet samples were analysed but after that period and due to the low levels found, in the period 1992-1995 only 2 total diets per year were analysed.

Only in 5% of the 4608 possible food group/pesticide combinations were concentrations above the limit of determination and except for lindane, levels found were always very low. DDE occurred most frequently followed by lindane and dieldrin.

Lindane concentrations in most samples of the bread group were below the limit of determination (1 µg/kg) and never above 3 µg/kg. However, in 2 samples collected between March 90 and March 91, high amounts of this substance were detected due to a fraudulent use of this pesticide in a local bakery. After taking the appropriate measures, the levels of lindane in the bread group were again very low or below the limit of determination and intakes estimated after 1992 fell from 0.5% to 0.1% of the TDI (Figures 12 and 13).

Dietary intakes of lindane, dieldrin, endosulfan and DDT are very low, always below 7% of the appropriate TDI (Figure 13) and in comparison with intakes in different countries, they are also very low (Figure 14). As expected, food groups of animal origin show more frequently organochlorine residues (Figure 15).

Organophosphorus pesticides residues

Organophosphorus pesticides have been analysed in 5 food groups (bread, cereals, potatoes, vegetables and fruits) of the 12 total diets collected in 1994 and 6 collected in 1995. 25 organophosphorus pesticide residues have been analysed (acephate, azinphos-ethyl, azinphos-methyl, chlorfenvinphos, chlorpirifos-ethyl, chlorpirifos-methyl, diazinon, dimethoate, ethion, fenitrothion, phenthotoate, fonofos, phosalone, phosphamidon, heptenophos, malathion, methacrifos, methamidophos, methidithion, omethoate, parathion-ethyl, parathion-methyl, pyrazophos, pirimiphos-methyl and quinaldars).
Of the 2250 pesticide/food group possible combinations, only 2% gave values above the limit of determination. 11 organophosphorus pesticides have been detected above the limit of determination but 4 of them (omethoate, acephate, chlorpyrifos-ethyl and methidathion) only on one occasion and therefore an intake has not been estimated. The intakes of the remaining 7 (chlorpirifos-methyl, pirimiphos-methyl, dimethoate, methamidophos, diazinon, phosalone and malathion) are well below the appropriate TDIs (less than 2.2%) (Figure 16).

Also, in 1995 and in order to identify the food item which was responsible for the presence of the residues detected in the group, the individual items of the vegetable and fruit groups which gave residues above the limit of determination, were analysed separately. Results confirmed that residues were present at very low levels in all the particular foods, and always below the appropriate MRL.

Dietary intakes of organophosphorus pesticides are comparable to those obtained in other countries in similar studies (Figure 17). Pirimiphos-methyl has been detected virtually on every occasion in the bread and cereals groups, sometimes at high levels if compared to MRLs established for cereal grains. These residues are present mainly due to postharvest treatment but account for only 0.5% of the TDI (Figure 16).

### Carbamate pesticide residues

17 compounds (aldicarb, aldicarb sulphone, aldicarb sulphone, bendiocarb, carbaryl, carbofuran, 3-hidroxi-carbofuran, ethiofencarb, ethiofencarb sulphone, ethiofencarb sulphoxide, methiobarb, methiobarb sulphone, methiobarb sulphone, methomyl, oxamyl, propoxur and thiophanox) have been analysed in 3 food groups (potatoes, vegetables and fruits) of 14 total diet samples collected between November/92 and December/93.

Residues were present in 0.9% of the determinations. Only 4 pesticides have been detected above the limit of determination (ethiofencarb sulphone, methiobarb, methiobarb sulphone and methomyl) and one of them (ethiofencarb sulphoxide) only on one occasion, so no intake has been calculated. Intakes of methiobarb and methomyl account for very low percentages of the appropriate TDIs, always below 1.2% (Figure 18).

There are few estimations of carbamate intakes in other countries and intakes in the Basque Country are similar to those of the USA. As regards the 3 groups in which these pesticides have been determined, vegetables show the highest percentage of detected residues (67%) whilst no residues have been found in potatoes. Although these compounds are widely used in this food item, the most persistent compounds are used in treatments prior to sowing and also it has to be kept in mind that the analysis are conducted on cooked potatoes.
Dithiocarbamate pesticide residues were analysed in potatoes, vegetables and fruits of 12 total diets collected between April/92 and April/93. Values above the limit of determination were only found on one occasion. It was a sample of the fruits group which had 0.14 mg/kg of CS$_2$. This is the reason why no intake has been estimated for this group of pesticide residues. The maximum intake which would correspond to this value would be 53 µg/day which is less than 4% of the most restrictive value of TDIs for the compounds in this group (ferbam and ziram). In this area the possibility has been considered of determining ethylenethiourea (ETU) from ethylene bisdithiocarbamates (EBDC) in total diet samples. Although, ETU determination is very interesting from the toxicological point of view, difficulties in its analysis have prevented us from accomplishing this project. Also, due to the low levels of CS$_2$ detected in total diet samples, the surveillance of these pesticides is limited at present to products where there is evidence of their wide and/or incorrect use (mainly leafy vegetables).

Once stated that dietary intake of pesticide residues is very low, the surveillance of these compounds is approached through selective controls in particular foods. In order to prioritise the food items which are to be included in the controls, several factors have been taken into account. First, the importance of those food items in relation to the total diet, second the dietary habits related to the food item (if it is consumed fresh or cooked) and finally, the existence of particular problems associated to certain crops.
Dithiocarbamate pesticide residues in lettuce

Selective controls on pesticide residues, were started in the Basque Country in 1993. The first one included the determination of dithiocarbamate residues in lettuce. Of the 66 samples analysed, 4.5% contained residues at levels above the MRL and 18% had residues but at levels below the MRL (Figure 19).

Pesticide residues in bread

On many occasions pesticide residues were found in the bread group and therefore, it was decided to conduct a selective control on this food item.

Residues of lindane, hexachlorobencene, α and β endosulfan, chlorpirifos, chlorpirifos-methyl, malation and pirimiphos-methyl were determined in 82 samples (69 of white bread and 13 of wholemeal bread) collected in the Basque Country. As expected, there were more residues detected in wholemeal bread than in white bread (23% in white bread and 69% in wholemeal bread) although levels found were always very low. In particular, residues of lindane (13 µg/kg), chlorpirifos (10–24 µg/kg) and pirimiphos-methyl (100–218 µg/kg) were found.

Pirimiphos-methyl is the pesticide residue most frequently detected and the one that appears in highest amounts in bread. Although there are not MRLs of pesticide residues in bread stated in the Spanish regulations (only in grain), the Codex Alimentarius Commission has set a MRL of 0.5 mg/kg of pirimiphos-methyl in white bread and of 1 mg/kg in wholemeal bread. Residues detected have always been well below this values.

Polychlorinated dibenzodioxins and dibenzofurans (PCDD'S and PCDF'S) and polychlorinated biphenyls (PCB's)

In order to estimate dietary intake of PCDD's and PCDF's by the population of the Basque Country, a total of seventeen 2,3,7,8 chloro substituted compounds have been determined in the groups which are more prone to contain them: eggs, meat (including meat products), fish, milk and dairy products and fats and oils. Determinations were conducted in 8 total diet samples collected...
between March 1994 and February 1995. The average intake of PCDD's and PCDF's in terms of 'Toxic Equivalents' was estimated to be in the range between 84 and 128 pg TEQ/day which is equivalent to 1.2–1.9 pg/kg body weight for an average weight of 68 kg. This estimation does not include the toxic equivalents of dioxin-like PCBs in order to have data comparable with other studies (the joint evaluation is conducted in the PCBs section).

Few countries have conducted estimations of global exposure to dioxins through the diet and they have not always used total diet studies. The intake estimated in the Basque Country is similar to that from other countries (Figure 21). As in the United Kingdom, The Netherlands, Canada and the USA, the largest contribution to the total intake of dioxins is made by milk and dairy products. After, this group come fish and meat. Eggs contribute to a very small extent (Table 5).

Regarding the risks associated with this level of exposure, a Tolerable Daily Intake (TDI) of 10 pg/kg body weight for 2,3,7,8-TCDD was recommended by an expert group convened by the World Health Organisation in 1990. Average dietary intakes estimated in other studies as well as our own estimations are below this TDI. However, the US Environmental Protection Agency (EPA) considering its carcinogenic potency and on the basis of a lifetime upper-bound risk of increased incidence of cancer of $10^{-6}$ for an adult, has established a maximum intake of 0.006 pg/kg weight/day. At present the EPA is conducting an exhaustive revision of the toxicity of dioxins which will be published soon. Nevertheless, there is an agreement among experts about the necessity to reduce potential contamination sources in order to avoid new discharges into the environment.

- Average intake of dioxins and dioxin-like PCBs (77, 126, 169, 105 and 108) is 443 pg TEQ/kg of which 315 are due to PCBs.
- This value corresponds to an average intake of 6.5 pg TEQ/kg body weight and it is therefore below the WHO tolerable daily intake of 10 pg/kg body weight/day.
- The highest concentrations of dioxins and PCBs are found in fish but the group which contributes most to the total intake is the milk and dairy products (40%). Fish and meat account for 27% and 23% respectively while fats and eggs provide only 8% and 2% of total intake respectively.
- The risk evaluation of dioxins and dioxin-like PCBs considered together is recent and there are few data with which to compare the results obtained in the Basque Country. However, the high contribution of PCBs to the total equivalents make it wise to maintain the surveillance in this area.

In order to evaluate the exposure to polychlorinated biphenyls (PCBs) through the diet, 11 PCB congeners have
been determined in the same samples as those selected for dioxin analysis. The congeners included are 3 non-ortho substituted (77, 126 and 169) and 2 mono-ortho (105 and 118) which have dioxin-like activity and for which toxic equivalency factors are available. The remaining 6 PCBs represent some of the most abundant in foods (28, 52, 101, 138, 153 and 180) (Table 6).

Non-ortho PCB concentrations found were broadly similar to those reported from a study of foods in the Netherlands\textsuperscript{65}. Reported congener-specific data for other PCBs in foods are remarkably variable, but results are of a similar order of magnitude to other reports\textsuperscript{74,75,76}.

It has not been until quite recently that a suitable range of analytical standards have become available and therefore, until now exposure to these contaminants used to be expressed as total PCBs intake. This approximation does not take into account the different toxicity of the various congeners and it is affected by the calculation method. Also, there is not a generally accepted toxic reference value for exposure to total PCBs\textsuperscript{73} which compromises the risk evaluation.

There are few published data on intake of individual isomers through the diet and the determination of the sum of toxic equivalents of dioxin-like congeners is particularly important. The average intake of these PCBs (77, 126, 169, 105 and 118) expressed as toxic equivalents per day is 315 pg, this is 4.6 pg/kg body weight for an average weight of 68 kg (Table 7).

The intake of this group of PCB congeners is nearly three times higher than that of the 17 dioxins and dibenzofurans, all expressed as toxic equivalents (315 pg/day against 128 pg/day). The average intake considering all the dioxins and dioxin-like compounds, is 443 pg/day which is equivalent to 6.5 pg/kg bw/day. Although the highest concentrations are found in fish, the milk and dairy products group due to its high consumption is the main contributor to the total intake of toxic equivalents (40%). The relative contribution of the other groups is as follows: fish 27%, meat and meat products 23%, fats and oils 8% and eggs 2% (figure 22).

The average daily intake in the Basque Country is 6.5 pg/kg body weight and it is below the Tolerable Daily Intake established by WHO\textsuperscript{68} (10 pg/kg bw/day). However, this intake accounts for 65% of the reference value and therefore it is necessary to maintain the surveillance in this area particularly that of PCBs with dioxin-like activity.
Mycotoxins: Aflatoxins

Aflatoxin M₁ was determined in the milk and dairy products groups of 19 total diet samples collected between March 1990 and December 1991. All concentrations were below the limit of determination (0.01 µg/L in the milk group and 0.025 µg/kg in the dairy products group) except for one sample of the dairy products group which contained 0.025 µg/kg. The total intake of aflatoxin M₁ was not calculated owing to these low levels detected.

Aflatoxin M₁ intakes have been determined in very few countries. In the UK duplicate samples of 10-week-old bottle-fed infants entire diets were analysed between July 1979 and February 1980 but the amounts detected were too low to allow for quantification of intake.

The Joint FAO/WHO Expert Committee on Food Additives regarded aflatoxins as potent human carcinogens but considered that there was not enough information to establish a tolerable level of exposure. Nevertheless, they recommended that dietary intakes should be kept to a minimum so as to reduce potential risk.

Selective Controls

Aflatoxin M₁ in milk

In 1990, a selective control of aflatoxin M₁ in milk was conducted. 61 samples of raw milk were collected at different farms throughout the Basque Country and 33 UHT samples were obtained from retail outlets.

- All the determinations of aflatoxin M₁ in total diet samples (except for one that had 0.025 µg/kg) were below the limit of determination.
- Aflatoxin M₁ levels both in raw and sterilised milk samples were low, never above the most strict tolerable levels (0.050 µg/kg).
- After a selective control of aflatoxin B₁, B₂, G₁, and G₂ in nuts, 3 peanut samples and 1 of pistachio nuts gave levels above permitted, although concentrations in peanuts were very close to the limit.
The levels of aflatoxin M1 were very low both in the raw milk and in heat treated samples, with values below the limit of determination in 80% and 85% of the samples included respectively. No sample gave values above 0.04 µg/kg. (Table 8)

Aflatoxins in nuts

A selective control of aflatoxins in nuts (pistachio nuts and shelled and unshelled peanuts) was conducted in 1993. 2 kg samples (made by four 500 g sub-samples) were collected directly from warehouses.

4 samples of the 61 included had residues of aflatoxin above permitted levels (10 µg/kg for total aflatoxin and 5 µg/kg for aflatoxin B1).

Radionuclides

The estimated average annual doses to the Spanish population from different sources is 3.5 mSv of which 2.4 mSv (70%) comes from natural sources (Figure 23). These data differ from those of the UK where about 87% is due to natural radiation. This difference can be attributed to the contribution of medical uses to the total dose which in the UK accounts for 14% whereas in Spain it is 30%.

Radionuclides were included in the total diet study in 1994/95 and they were analysed in 8 total diets collected between January/94 and April/95. Radioisotopes were selected depending on their origin, half life and available resources and in the end it was decided to include 4 artificial radionuclides (\(^{89}\)Sr, \(^{90}\)Sr, \(^{137}\)Cs, \(^{134}\)Cs) and one natural (\(^{40}\)K). Caesium and strontium radionuclides are indicators of human activity and therefore they are the first analysed in situations of exceptional emissions. Gamma emitters (\(^{137}\)Cs, \(^{134}\)Cs and \(^{40}\)K) were determined in the 16 food groups of the total diet whereas beta emitters (\(^{89}\)Sr, \(^{90}\)Sr) were only analysed in 4 food groups (fish, milk, dairy products and vegetables).

Intakes can be calculated by combining the activity concentrations in the particular group with consumption data. Activity concentrations have to be previously transformed into doses using Dose coefficients which are stated in the legislation. Finally, daily intakes are converted into annual intakes to enable comparability with data from other countries and with the annual dose estimations carried out by the ‘Consejo de Seguridad Nuclear’ (CSN). Estimated doses are shown in Table 9.

The levels of specific activity for the artificial radionuclides included (\(^{89}\)Sr, \(^{90}\)Sr, \(^{137}\)Cs, \(^{134}\)Cs) are very low, almost always below the limit of determination. Annual dose equivalents by ingestion
of $^{137}\text{Cs}$ and $^{90}\text{Sr}$ are similar to those estimated for countries situated quite far away from Chernobyl like the UK or Japan (Figure 24). Regarding intake of natural radionuclides, the only estimated intake has been that of $^{40}\text{K}$. This isomer is the highest contributor to internal radiation and the estimated value obtained through the total diet study is above the approximate estimation carried out by the CSN (180 µSv/year for the Spanish population). However, the value is included within the normal range.

Estimated radionuclide intakes for the Basque Country account for levels below 0.03% of the annual limit intakes stated in the Spanish Regulations (Table 10).

As regards derived limits in foods, there are not such references in the Spanish legislation but if the results of the Basque Country are compared to derived limits published by the National Radiological Protection Board (NRPB) of the UK, it can be observed that the highest concentration found which is that of $^{90}\text{Sr}$ in a sample of the dairy products group (0.23 Bq/kg), accounts for 0.8% of the derived limit for this radionuclide in milk. Also, the highest $^{137}\text{Cs}$ concentration detected in a sample of the fish group (0.57 Bq/kg), accounts for 0.19% of the most restrictive level stated for this radionuclide (in milk, because there is not a Derived Emergency Reference Level for $^{137}\text{Cs}$ in fish).

Finally, it has to be taken into account that there is a large number of samples with less than the limit of detection results and therefore, findings may not be taken as conclusive. However, milk and dairy products, fish and meat are the main contributors to $^{137}\text{Cs}$ intake and they account for 29%, 14% and 14% of the total intake respectively. For $^{90}\text{Sr}$, milk is the main source (50% of total intake) and $^{40}\text{K}$ is widely distributed among the different food groups, the main sources being fruits (20%), meat and meat products (16%) and dairy products (15%).

- Total annual dose due to artificial radionuclides ($^{90}\text{Sr}$, $^{99}\text{Sr}$, $^{137}\text{Cs}$, $^{134}\text{Cs}$) is very low even assuming values below the limit of determination equal to the limit.

- Estimated intakes account for very low percentages (below 0.03%) of the Annual Limit Intakes stated in the Spanish Regulations.

- Data obtained by surveillance in 1994/95 as well as the Total Diet Study provide a permanent infrastructure which can be used as a reference to evaluate trends and changes in the future.
Nitrate and Nitrite

Dietary intakes of nitrate and nitrite have been determined in 3 groups (potatoes, vegetables and meat products) of the total diet in the period 1992–1995.

Nitrate

Dietary intake of nitrate in 1992–1995 ranged from 50 to 64 mg/day which accounts for 20–26% of the ADI (Figure 26). Average intake in that period was 60 mg/day. Nitrate concentrations in most drinking waters of the Basque Country are low, around 5 mg/L. Assuming a daily intake of 1 L of water, it would mean an additional 5 mg of nitrate giving a total daily intake of 65 mg, which accounts for 26% of the ADI.

Dietary intake of nitrate in the Basque Country is similar to those estimated in other countries like the United Kingdom\(^{105}\) and Finland\(^{106}\) both at 54 mg/day. The highest intakes have been estimated in the Netherlands\(^{23}\) (84 mg/day) where the contribution of drinking water to the total intake was included (Figure 27).

As expected, the vegetable group is the main contributor to the nitrate intake and it accounts for 75% of the total. Potatoes contribute 12% and meat products, 5%. The remaining 8% originates from drinking water.

In an attempt to identify the vegetables which had highest nitrate concentrations and to see how nitrate concentrations changed with the time of the year, the individual vegetables included in the 12 diets of 1995 have been analysed separately (Figure 28). Chard, spinach and lettuce gave the highest levels.

Nitrate concentrations in spinach and lettuce sampled for the total diet study have been compared to levels set in the EU proposal for a Regulation where maximum permitted contents for nitrate in lettuce and spinach are set\(^{108}\). Some spinach samples have concentrations above the proposed limit (Figure 29) whereas lettuce content was always below the maximum content proposed for this vegetable (Figure 30). There are no proposed levels for nitrate content in chard but levels found in this vegetable were the highest of all the products analysed with an average of 3200 mg/kg and up to a maximum of 6300 mg/kg (Figure 31).

Apart from the effect of nitrate Community limitations on improvement of the quality of vegetables, surveillance programmes to progressively reduce nitrate levels in certain species have to be also considered. Codes of Good Agricultural Practices to adequately use fertilisers...
as well as selection of varieties which accumulate less nitrate will help to accomplish this objective.

Nitrite has only been detected above the limit of determination of the analytical technique (0.1 mg/kg) in the meat products group. Nitrite intake ranged between 0 and 0.2 mg/day which accounts for a maximum 2% of the ADI for nitrite ion.

As regards nitrite intake estimations carried out in other countries, the estimated intake in the Basque Country is among the lowest reported in the literature and the same as that estimated for the Netherlands\textsuperscript{107}. In the United Kingdom\textsuperscript{105} and Finland\textsuperscript{106} higher intakes have been estimated (Figure 32).

Selective Controls

Nitrate and nitrite in meat products

Nitrate and nitrite content were determined in 'chorizo', 'chistorra' and 'lomo adobado' (these are 3 meat products, the first 2 of them are a kind of cured sausage which is consumed as such or cooked and the third one is pickled pig loin which is consumed cooked) and the control showed an incorrect use of these additives in a large number of samples (Table 12).

Nitrite was only present in 8 of the analysed samples, 7 of which were 'lomo adobado' and one was 'chorizo'. Maximum permitted levels were only exceeded in 2 samples (Table 13).

Nitrate content in 24 'additive preparations' intended for meat products production has also been determined and the label information was also studied. The evaluation of

- The average estimated nitrate intake in the Basque Country including drinking water is 65 mg/day, which accounts for 26% of the ADI.
- Vegetables are the main source of nitrate in the diet whereas meat products contribute only 5% and water 8%. Chard, spinach and lettuce are the products which have highest nitrate content. Nitrate content in spinach is above levels permitted in the EU proposal.
- Meat products are the only source of nitrite in the diet. Nitrite intake is very low and it is below 2% of the ADI.
- Nitrate and nitrite are used incorrectly in the elaboration of meat products ('chorizo', 'chistorra' and 'lomo adobado'). Therefore, several measures have been adopted, and their effectiveness will be evaluated in the course of new controls.
results has highlighted several irregularities but the reason for the higher than permitted levels present in these products is mainly attributed to misuse of 'home-made' producers.

The control of nitrate and nitrite levels in meat products have shown that their addition to these products by home-made producers is not done correctly. Therefore, informative and repressive measures have been adopted, the effectiveness of which will be evaluated in the course of new controls.

**Food additives**

**Sulphite**

Sulphite has been determined in 7 food groups of the total diet where its addition is permitted. These are meat products, fish, dairy products, cereals, sugar and preserves, non-alcoholic beverages and alcoholic beverages. The estimated sulphite intake in 1995 was 19-24 mg/day of SO₂, which accounts for 8-10% of the ADI (3.5 mg/kg body weight). This intake is similar to that estimated in other countries (Figure 33). However, the only groups that gave values above the limit of determination were meat products and alcoholic beverages and therefore, the estimation of 19 mg/day which assumes values below the limit of determination equal to zero appears to be more realistic. Alcoholic beverages contribute 75% of the total intake of sulphite.

**Selective Controls**

Selective controls on sulphite are carried out regularly on 2 types of products: meat products and crustaceans (where the presence of boric acid is also looked at).

**Sulphite in meat products**

Sulphite in 'home made' meat products have been controlled through inspection and sampling at several butchers in the Basque Country. In 1994, and after collecting samples of minced meat, fresh sausages and hamburgers it was evident that their use was incorrect (Table 15).

Actions taken are mainly focused on informing and assessing the producers, notwithstanding the discouraging effect that always have sanctions. Further selective controls will serve to evaluate the effectiveness of the measures adopted.
Sulphite and boric acid in crustaceans

Boric acid was detected in 29% of fresh shrimps, 33% of frozen shrimps and 17% of frozen prawns. As regards sulphite, in 1994 20% of frozen shrimps and 33% of fresh shrimps samples had levels of SO₂ above 400 mg/kg. Moreover, the extent of use of this additive was further demonstrated when it was confirmed that 44% of frozen crustaceans and 55% of fresh crustaceans analysed, gave SO₂ levels in the range 150-400 mg/kg. Once adopted Directive 95/210, manufacturers will have to readjust dosages of the additives used.

Nutrients

The nutrient intakes estimated from the Basque Food Survey conducted in 1990, suggested deficient intakes of iron and zinc in certain population groups. This was the reason why, these elements were included in the total diet study. As regards selenium, the apparently small margin between required and toxic levels made it wise to determine its content in foods (Table 17).

Iron

Average dietary intake of iron in the Basque Country is 11.3 mg/day. This element has been determined in the 16 food groups of 14 total diet samples collected between March/90 and June/91. As shown in Table 17, recommended iron intakes in Spain are 10 mg/day for males and 18 mg/day for females120 and therefore, although male requirements would be satisfied, female intakes would only account for 65% of the recommended value.

Iron intake by females does not even reach lower recommended allowances like those given by the National Research Council124 (15 mg/day for females) or the United Kingdom122 (14.8 mg/day for females).
As shown in Figure 34, average dietary intake of iron is low in comparison to other countries like Finland (17.3 mg/day), Sweden (16 mg/day) and China (22.7 mg/day), and it is closer to iron intake in the UK (10.3 mg/day) or the Netherlands (12.1 mg/day).

As regards contribution to total iron intake, meat and meat products were the main contributors (28%) and as in this group iron is mostly present as haem-iron, its bioavailability is high. Bread and cereals are next in importance (19%) followed by alcoholic beverages (14%). Eggs, fish and pulses and nuts gave high concentrations of iron but due to their lower consumption they do not contribute substantially to the total intake (Figure 35).

**Zinc**

The mean daily dietary intake of zinc was 11.6 mg/day and it accounts for 77% of the recommended intake for Spain (Table 17). There is a wide variation between recommended intakes established in different countries and therefore, it is not always easy to determine whether there is a deficiency or not (Table 16). For instance, recommended intake in the UK is 9.5 mg/day for males and 7 mg/day for females whereas in the USA, it is 15 mg/day for males and 12 mg/day for females\(^1\) whereas in the USA, it is 15 mg/day for males and 12 mg/day for females\(^1\) and in Spain 15 mg/day\(^1\).

In the Basque Country, serum zinc was determined in a group which participated in the Food Survey and sub-optimum values were only detected in 5% of females between 25-34 years\(^5\). Therefore, although zinc intakes are below recommended values, the fact that serum zinc levels are normal for the majority of the population confirms the idea generally assumed that zinc recommendations are somewhat overestimated.

Average dietary intake of zinc is similar to that estimated in other countries (Figure 36).

Schumacher et al.\(^133\), have conducted a duplicate diet study in Tarragona (Spain) and have estimated an intake of zinc of 6.8 mg/day. One reason that could explain the difference between their estimation and that for the Basque Country is that food consumption patterns can be changed in the course of this type of study and total amounts of foods and energy tend to be lower than those calculated by other methods\(^134\).

Half of the total zinc ingested comes from the meat and meat products group. Among the other groups, milk and dairy products (15%), bread and cereals (10%) and fish (9%) are worth mentioning. This means that 75% of the zinc is provided by foods from animal origin and therefore in a more bioavailable form than the zinc from foods of vegetable origin. Pulses and nuts contain high levels of zinc, but because they are consumed in low amounts, they do not contribute substantially to the total zinc intake (Figure 37).
Dietary intake of selenium in the Basque Country has been estimated through the determination of this element in the 16 food groups of the total diet study in the period 1992-1994. The average selenium estimated intake was 84 µg/day (Figure 38), 50% of which originates from fish and 30% from meat and meat products (Figure 39).

Dietary selenium intake estimated in the Basque Country was similar to the USA estimation and higher than intakes estimated in the Netherlands, Sweden, the Czech Republic and China (Figure 40). There is not a recommended intake for selenium in Spain, but this estimation is above those from other countries.

- The average intake of iron is 11.3 mg/day. This satisfies male iron requirements (10 mg/day) but not female’s (18 mg/day). Female iron intakes are therefore deficient and this is one of the most frequent nutritional imbalances in developed societies.

- Meat and meat products group is the main contributor to the total iron intake (28%) and because it is present as haem-iron, it is of high bioavailability.

- The average zinc intake is 11.6 mg/day and it accounts for 77% of the recommended intake. However, the fact that serum zinc levels in the majority of the Basque population are normal, confirms the generally recognised idea that recommended intakes for this element are overestimated.

- Half of the dietary zinc comes from the meat and meat products group. Foods from animal origin where zinc is of higher bioavailability contribute 75% of the whole diet.

- Average intake of selenium is 84 µg/day and it is above most reported selenium intakes in the literature. In Spain, there is not a recommended intake for selenium but the intake in the Basque Country is above recommendations for other countries.

- The main sources of selenium are fish (50%) and meat and meat products (30%).
Veterinary drug residues

The Health Department of the Basque Government together with the participation of other Institutions (local administrations of Bilbao and San Sebastian and the Agriculture Department) set the National Surveillance Programme in 1990. Thereafter, the scheme has been modified on several occasions in order to adjust it as much as possible to the real situation of this sector in the Basque Country. The most severe change took place in 1992 as a consequence of the clenbuterol outbreaks that occurred in January of that year due to liver consumption. Table 18 includes the various sampling criteria which have governed the surveillance of the different residue groups in the period 1990-1995.

Thyrostatic residues

In Spain, prohibition of thyrostatic agents for animal production dates back to 1977. In the Basque Country, the control of use of these substances has been conducted through the determination of residues in the thyroid gland which is the organ where the highest levels of this substances can be found after their administration to the animals. In the period 1990-1993, random sampling was carried out in slaughterhouses in order to determine thyouracils (thyouracil, methyl-thyouracil, propyl-thyouracil and phenyl-thyouracil) and mercaptoimidazol. The number of samples was based on the throughput for slaughterhouses. The majority of samples collected were of cattle, although in 1992 pig samples were also included. No residues were detected in any of the 16 pig thyroid glands analysed in this period.

The number of samples analysed in cattle was reduced from 1990 to 1993 (Figure 41) while the percentage of samples with residues was kept at similar levels in that period, the average value for the 4 years being 3%. The

- The percentages of samples with thyouracils and mercaptoimidazol residues in the period 1990-1993 were similar, on average 3%.
- Although the percentage is low, it shows that these substances are still being used for animal production.
substances detected were methyl-thyouracil, mercaptomidazol and propyl-thyouracil combined to phenyl-thyouracil. This results show that these substances are still being used for animal production and therefore it is necessary to keep up control on their use.

The first sign of the use of these substances on the animal is the hyperplasia of the thyroid gland and therefore, since 1993, a gravimetric procedure was used to screen thyroid glands. Those exceeding the threshold weight (50 g) were subjected to analysis. Carcasses were retained until results became available. In 1994 and 1995 only 2 samples were collected under these circumstances and no residues were detected.

Anabolic agents

Stilbenes

The control of the illegal use of stilbenes was carried out between 1990 and 1993 through the determination of residues in urine at the time of slaughter. At the beginning, this control was established for cattle but in 1991 pig samples were also included. No stilbene residues were detected in any of the 548 samples analysed in this period. These results agree with those obtained in other autonomic regions and European countries where no stilbene residues have been detected since 1986. In 1994, it was decided to discontinue the determination of stilbene residues giving priority to other substances.

Natural hormones

In 1990 and 1991 a sampling programme designed to find out the levels of these substances in animals was conducted. 183 samples of bovine serum were collected and levels of testosterone, progesterone and 17ß-oestradiol were analysed (Table 19).

Interpretation of the analytical results for natural hormones proved to be difficult because it is not generally

- Neither stilbene nor synthetic anabolic agent residues have been detected in any of the samples analysed.
- The control of the illegal use of natural hormones and synthetic anabolic agents is conducted through surveillance of implants from animals at slaughterhouses.
possible to distinguish administered hormones from those produced naturally by the animal. Therefore, in 1992 the analysis of natural hormones was ceased.

In 1994 the EU published a report which included reference values for natural hormones, that if exceeded, the investigations and measures foreseen in article 6 of Directive 85/358 should be carried out (Table 20). However, even if levels above those reference values were found, no legal action could be started because there are not maximum permitted levels in serum. Therefore, the fraudulent use of this substances can only be proved through surveillance on the farms.

**Synthetic anabolic agents**

To control the presence of trenbolone, zeranol and nortestosterone in urine of cattle and pigs, a sampling programme was conducted in 1990-1993 (Table 21). The results of surveillance revealed no evidence of misuse of synthetic anabolic agents.

**Antimicrobial and chloramphenicol residues**

The control of antimicrobial residues in meat producing animals was initiated in the Basque Country in 1990. In 1990 and 1991 random samples of muscle were collected for inhibitors and chloramphenicol analysis following directions stated in RD 1262/89. The number of samples was based on the throughput for slaughterhouses. The determination was carried out by a microbiological test which measures the ability of the sample to inhibit the growth of Bacillus cereus and that is associated with the presence of antimicrobial agents in animals. Chloramphenicol is determined separately by HPLC (Table 22).

In 1990 only samples of cattle were collected but in 1991 pigs were also included. The 2 samples which gave residues that year were of pigs. Due to the low number of cattle samples with residues and considering that in cattle, inhibitors are used in individual therapeutic treatments, in 1992 it was decided to start a control of inhibitors in cattle sent for urgent slaughtering. Carcass and offals were kept until analytical results became available. These measures tried to avoid the release for consumption of carcasses which contained antimicrobial residues, because it was assumed that animals sent for urgent slaughtering had high probability of being subject to recent treatment. Apart from that, random sampling on pigs and cattle was still conducted but sulphonamide residues were also determined in these samples by HPLC. The reason for determining the sulphonamides separately was that the microbiological assay has low sensitivity in comparison to the MRL for these substances (100 µg/kg). Chloramphenicol determination in random sampling was discontinued in 1992 but was still carried on in the target sampling. This was also done in 1993.

Figure 43 shows the results obtained between 1992 and 1995 for the target sampling in cattle. Although in 1993 the number of samples collected dropped considerably, the number of
samples with residues of antimicrobial agents was somewhat higher than the previous year. However, the percentages obtained were not as high as expected. This could be attributed to limitations of the microbiological technique to detect some inhibitors and/or to an incorrect selection of the target samples.

In 1994, the criteria for random sampling were modified. From this year onwards it was decided that target samples were those that underwent urgent slaughtering but which showed evidence of treatment with antimicrobial agents. Also, animals which in ante-mortem or post-mortem inspections showed pathologies that were prone to have been treated with antimicrobial agents were included.

Figure 43 shows that the selection of target samples at slaughterhouses has improved since 1992, particularly in 1995 when more than 50% of the samples had residues of antimicrobial agents. 78% of the samples had been classified as target samples based on the ante-mortem inspection and included animals which had suffered digestive, respiratory or post-labour processes.

As regards the random sampling, since 1992, most of the samples that contained residues of antimicrobial agents or sulphonamides were samples of pigs (Table 23). This was the reason why since 1994 the random sampling to determine antimicrobial residues and sulphonamides was carried out only on pigs.

Results show that the use of sulphonamides in pigs is a common practice. 17% of the total number of pig samples analysed in 1992-1995 had sulphonamide residues although only 4% had levels above the MRL (100 µg/kg). This is, of the 46 pig samples which showed sulphonamide residues, 12 had levels above the appropriate MRL. All of them contained suphamethazine which on 5 occasions was accompanied by sulphamethoxypiridazine (Figure 44).

In order to obtain more information about conditions of use of sulphonamides in pigs, in 1995 target sampling was conducted based on the results of previous years. Farms from which animals with residues of sulphonamides came,

• The use of antimicrobial agents in cattle is limited to individual therapeutic use in infectious diseases.
• The target sampling of animals that appear to have gone through a therapeutic treatment is a good approach to assessing the presence of antimicrobial residues in cattle.
• Sulphonamides are widely used in pig production.
• It is necessary to investigate in farms, whether generalised administration of sulphonamides in pigs is carried out according to legal requirements.
• Even when residues are present due to therapeutic use, it is necessary to maintain the withdrawal periods so as to avoid the presence of residues in meat.
were included in a list which was delivered to all slaughterhouses. When a batch of animals from any of those farms entered the slaughterhouse, 10% of the animals was sampled up to a maximum of 8 animals per batch. This was done on 13 batches with a total of 54 animals sampled. 31% of the batches showed residues of sulphonamides. It was also noted that levels of sulphonamides were similar in animals which came from the same farm and on some occasions, levels found were well above the MRL (up to 5200 µg/kg). This suggested generalised treatments of all the animals coming from a certain farm.

Most of the farms whose animals showed sulphonamide residues were located outside the Basque Country and therefore they could not be visited to definitely establish the reasons for the presence of residues. Anyway, and even assuming that sulphonamide administration was done with a therapeutic aim, it is important that withdrawal periods be followed to avoid the presence of residues at levels above the MRL. This is the only way that will guarantee the protection of consumers against undesirable levels of sulphonamides in pork and pork products.

**Clenbuterol and other β-agonists**

In the Basque Country, clenbuterol residues are controlled since 1990. In 1990, random sampling of cattle urine was conducted in the slaughterhouses to comply with the National Surveillance Programme. Only 1 of the 50 samples analysed showed residues, although as later proved it was a generalised practice.

In 1992, clenbuterol poisoning due to human consumption of liver took place and several families were affected (Table 24). As a result, an intensive surveillance scheme to control clenbuterol was initiated. Urine samples were collected at slaughterhouses and liver samples at refrigerated storehouses. Sample collection was always accompanied by retention of the animals and/or incriminated products until analytical results were available. Carcasses or products which did not show residues of clenbuterol were delivered for consumption and only those products that contained residues were retained. Altogether, more than 1000 samples were analysed between January and March 1992 (Table 25).

After April 1992, a strategy to control clenbuterol and other β-agonists residues in animals was designed and this included the determination of residues in samples from farms, slaughterhouses and refrigerated storehouses. The sampling included random and target samples. Target samples were based on the origin of the animals or on ante-mortem or post-mortem inspection and required that livers were retained until the result became available. If no clenbuterol residues were detected they were finally released to the market. It has to be considered that only 51% of the cattle consumed in the Basque Country is slaughtered in slaughterhouses from the Basque Country and of this, 27% is from farms placed in this area.

Apart from clenbuterol, other β-agonists like mabuterol, cimaterol, clenproperol, salbutamol, and terbutaline have been included and also other animal species like pigs and ovine.
As shown in Figure 45, the number of samples analysed increased yearly except for 1993 because the number recorded for 1992 included the samples collected under the intensive clenbuterol scheme.

As regards the percentage of samples taken according to the different criteria for sampling, it can be seen that after 1992, the percentage of target samples decreased. This was considerable in 1994 and 1995 but hardly noticeable between 1992 and 1993.

Trends in the number of samples taken per year (Figure 45) and in the percentage of samples taken by each criteria (Figure 46), are a consequence of the analytical results obtained (Figure 47). The number of samples increases yearly because clenbuterol residues had still been detected the previous year. The reduce in target samples collected after 1994 reflects the reduce in the number of samples that contained clenbuterol residues.

After April 1992, the number of positive samples with clenbuterol residues and the levels found decreased. This decrease was probably due to the strict control measures imposed as a consequence of the outbreak. But in 1993, when the control measures had been softened, an increase in the number of samples with residues was observed. However, the levels found were not very high. This fact appears to indicate that the use of clenbuterol in animal production has not been abandoned but that the continuous surveillance had led to a more controlled administration: farmers control better the doses used and also the withdrawal periods.

Another point that backs this idea is the trend in the percentage of samples with residues depending on the sampling place (Fig 48). In 1992, 60% of the samples that contained clenbuterol residues had been taken in farms and slaughterhouses. There, urine samples were taken to investigate the presence of clenbuterol. In 1993, the percentage decreased to 30%. On the other hand the percentage of samples with residues from refrigerated storehouses where liver samples were collected, increased from 40% in 1992 to 70% in 1993. This can be attributed

- Clenbuterol is widely used as growth promoter in animal production.
- The control measures have forced a reduction in the number of samples with clenbuterol residues and in the levels of clenbuterol detected.
- Regardless the control measures carried out, clenbuterol is still being used in animal production but the trends in the results since 1992 suggest a more controlled although fraudulent use of this substance.
to the fact that clenbuterol residues in livers can be detected 14 days after administration whereas in urine, 4 days after the administration it is not possible to detect the residues with the analytical techniques available.

In July 1994 clenproperol was detected in 8 urine samples three of which contained also clenbuterol. Also, cimaterol, mabuterol, salbutamol and terbutaline were determined in 90 urine samples but no residues were found. That year, the percentage of samples with clenbuterol residues decreased dramatically and as already mentioned it was probably due to a more controlled although fraudulent use by farmers.

This situation makes it necessary to look for more efficient control measures and more sensible methods of detection. In 1995 the control scheme included determination of residues in retina because in this tissue clenbuterol remains longer after treatment. This strategy was used in all the Autonomic Regions.

In 1995 β-agonists were also determined in 48 samples of ovine urine and in 16 samples of pigs urine. No residues of any of the above mentioned β-agonists were detected.