i'Tec_T6
May 2003

Keywords: Carry-over, Homogenisation, Additives

Comparing internal tracers/external tracers

A mix component referred to as a **tracer** is used to assess mix homogeneity or the level of carry-over at industrial sites. There are two types of tracer:

- internal tracers: a formula ingredient
- external tracers: an external agent supplementary to the formula

The methods used to select a tracer to assess mix homogeneity or carry-over at production lines have been discussed in the technical datasheets that describe this kind of assessment (i'Tec_T2 and i'Tec_H1). The strict application of these rules and, in particular, the criterion for inclusion in the positive list, is currently making it impossible to perform such tests. Given the current emphasis within France placed on medicated feeds under Directive 95/69, some veterinary officers-inspectors, and some plants, have resorted to using drug substances to carry out these assessments.
Against this background, this datasheet presents some examples of comparative results between these two types of tracer and lists the pros and cons of their respective usage.

1. Comparison of the results

A few comparisons of the results obtained at Tecaliman with both types of tracer during the same set of tests are given below.

1.1. Homogeneity

1.1.1. General database

The database developed by Tecaliman since 1993 (I'Tec H4, 2001) has shown that internal tracers increase the risk of obtaining inter-sample variance that has no significance in relation to analysis performance (from sampling to analysis via sample processing), as evaluated by duplicating the analyses on each sample (Table 1). In other words, when using an internal tracer, inter-sample variability is more likely to derive from the analytical process itself rather than from mixer performance.

	Number of assessments	% of insignificant assessments
Internal tracer	62	53.2%
External tracer	130	44.6%

Table 1: Extract from Table 28 of the bibliography

Furthermore, a comparison of populations of results for homogenisation performance tests failed to detect differences between internal and external tracers. Lastly, external tracers help to improve feed safety as, in many cases, they seem to draw attention to the appearance of possible dispersion issues (e.g. Figure 1).

1.1.2. Test repeatability

In 1999, both internal and external tracers were used during tests run to assess the repeatability of homogenisation performance tests on industrial mixers (Table 2 – I'Doc H2, 2000).

Site	Matrix	Tracers	$CV_{homogeneity}$			Mean
I	Broiler feed	RF-red lake	1.8	0.0	0.0	0.6
		Brilliant blue	7.6	0.0	8.0	5.2
		Lasalocide	3.3	2.9	1.4	2.5
J	Broiler feed	RF-blue lake	7.6	9.3	9.5	8.8
		Meticlorpindol	4.6	5.1	3.3	4.3
K	Rabbit feed	RF-blue lake	8.5	15.7	10.7	11.6
		Meticlorpindol	6.8	10.6	7.0	8.1

Table 2: Mean $CV_{homogeneity}$ obtained at three plants for various tracers after repeating the homogenisation tests

The results demonstrated that, in line with the internal tracers and despite the repeatability effect, the external tracers (microtracer and brilliant blue) made it possible to differentiate between a higher-performance mixer (I or J) and a lower-performance mixer (K). This was only possible, however, when mixers were compared using the same tracer.
Certain individual results also demonstrated, sample by sample, good alignment between an internal tracer (Meticlorpindol) and an external tracer (RF-blue lake microtracer) (Figure 1).

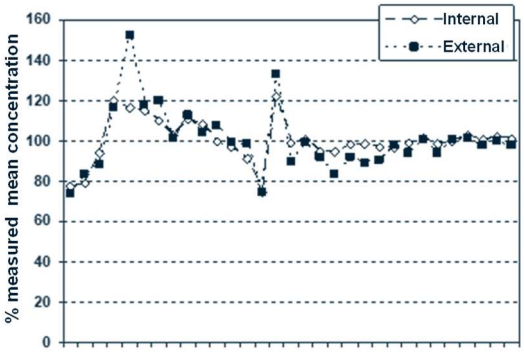


Figure 1: Change in concentration of two tracers in the samples taken at a mixer output

1.1.3. Research into tracers

Tests carried out jointly with the DGAL on which tracer to use when testing a mixer's homogenisation performance (I'Tec H7, 2003) showed that, depending on which tracer was chosen, a given mixer could be characterised by a total coefficient of variation that ranged between 2 and 29%. The choice of tracer is therefore a vital step in checking mixer performance, and has to be made based on a list of technical criteria among which analysis performance is an important factor.
Out of the 12 tracers tested (11 internal and one external), the external tracer used by Tecaliman (RF-blue lake microtracer) was the only one that guaranteed a test result that assessed the mixer's technical performance. The oxytetracycline used during these tests gave a higher coefficient of variation (6.6%) than that obtained with other tracers, including certain internal tracers in the same mix (Manganese: 2.4%, Meticlorpindol: 0.3%, microtracer: 2.6%). This suggests that the physical properties of oxytetracycline limited its dispersion. Qualifying the mixer with a 6.6% CV when it is capable of dispersing three other products with lower CV would therefore be incorrect and could have far-reaching consequences.

1.2. Carry-over

The results of carry-over tests list two successive collector batches, referred to as L3 and L4. Carry-over is expressed as a percentage of the tracer concentration measured in the last tracer batch.

1.2.1. Plant

Carry-over assessment repeatability tests were performed in 2000 as part of a program with the DGAL (i'Tec T8, 2003). The results from three plants showed that the mean carry-over evaluated by the external tracer could be similar to, or even greater than, those of the internal tracers (Table 3). This can be used to reliably detect carry-over hazards before they appear, and thereby improve feed safety.

		L3	L4
A	RF-blue lake microtracer	10.4	1.3
	Dimetridazole	8.8	0.4
B	RF-blue lake microtracer	4.8	1.1
	Oxytetracycline	6.4	1.5
C	RF-blue lake microtracer	7.6	2.0
	Oxytetracycline	3.9	0.4

Table 3: Mean carry-over in two successive collector batches produced at three industrial sites, repeated three times

Repeatability was also systematically enhanced when using the external tracer.
In another test, the variation in the size of collector batches revealed that carry-over from both types of tracer developed along similar lines, highlighting the same effect of the stimulated factor: carry-over durability increases as the size of the collector batches decreases (Figure 2).

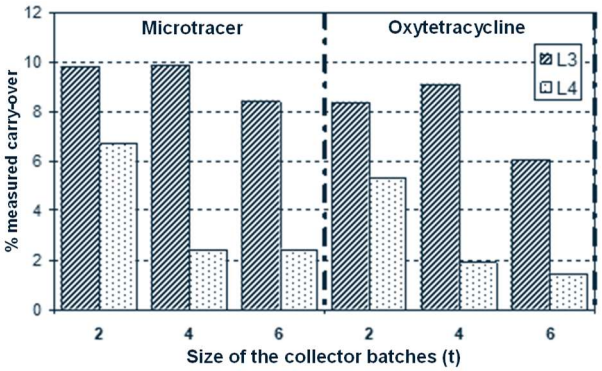


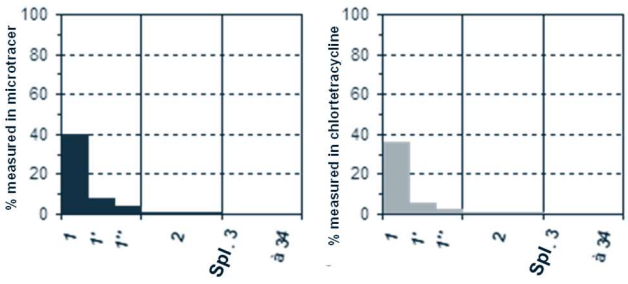
Figure 2: Carry-over in two successive collector batches produced by varying the size of collector batches from 6 to 2 t.

1.2.2. Trucks

In the latest Tecaliman study (i'Tec T8, 2003), interbox carry-over profiles, measured on 10 truck models, gave largely similar results between the tracers, while the external tracer (RF-blue lake microtracer) was compared to oxytetracyclines and chlortetracyclines in premixes of various brands (Table 4).
Again, carry-over profiles often showed significant similarities as shown in the example of truck D (Figure 3).

Trucks	Interbox carry-over (% measured)	
	External	Internal
A	0.55	0.65
B	0.12	0.12
C	0.15	0.05
D	0.61	0.45
E	1.41	1.41
F	0.14	0.18
G	0.14	0.15
H	0.02	0.04
I	0.02	0.08
J	0.14	0.17

Table 4: Mean interbox carry-over evaluated in 10 different trucks using two types of tracer



RF-blue lake microtracer Chlortetracycline

Figure 3: Change in the interbox carry-over profile in truck D using two tracers

1.3. Conclusion

All the tests indicated good alignment between the two types of tracer and the gains in feed safety provided by use of an external tracer: possible amplification of phenomena, improved repeatability of carry-over tests, guarantee of testing the equipment and not the product.

2. Internal tracers

2.1. Pros

- The substance used is that actually used at the plant under the same premix conditions.
- The tests can be performed up to the finished product stage, provided that the substance is stable with respect to the manufacturing process.
- Cons
- The use of drug substances deviates from Directive 95/69, which refers to certain substances on positive lists: A, D, J, certain vitamins and oligo-elements.
- Marketed drug products are not assessed in terms of their effective dispersion ability criterion. As such, if the aim is to test mixer performance, it is important that the product's galenic formulation does not create an unacceptable result that would be attributed to the mixer, while it is the product itself that has generated the result. In other words, it would be tricky to test an industrial process based on its ability to disperse in a feedstuff a product that is actually resistant to dispersion (see 1.1.3).
- High analysis costs limit the number of tests that an industrial can carry out aimed at making their production techniques safer (research into optimum homogenisation conditions, rinsing procedures, etc.).
- Analytical precision (homogeneity) and detection thresholds (carry-over) cannot be relied on to fit with the desired results.
- The small size of the test portions for this type of substance (a few grams) requires the use of adapted sampling and sample processing procedures capable of producing test portions that are representative of the samples taken on site.
- Changes in performance from one test to another can only be compared and tracked if the tracer's physical properties remain the same between any two tests. Use of an internal tracer should be avoided if the holder of the marketing authorisation is able to switch active substance supplier without notifying their client, and if their manufacturing process only consists of a dilution that does not alter the tracer's physical properties.
- When performing carry-over tests, using a medicated feed for tracer batches means having to:
  - Focus the assessment on a drug formulation that does not necessarily correspond to a feedstuff that is representative of the plant's main output (targeted species, meal/pellets, etc.).
  - Study a special feed production case for which the plant has planned special rinsing practices. The test then has to take account of these practices, which may deviate from current manufacturing practice for other feedstuffs.
  - Require a veterinary prescription (and, therefore, a farm) for a quantity of medicated feedstuff that is double the nominal size of the mixer, without which the feedstuff will have to be destroyed.
  - Produce two feedstuff batches of the same size, based on the same formula, which are "contaminated" by an unknown quantity of drug substance. As such feedstuffs cannot correspond to a proper feed manufacture, they are generally recycled or rerouted through the plant. In other words, performing the test using this method would effectively create the very risk that the assessment is supposed to control.
  - Perform a test with a premix incorporated at a minimum of 5 kg/t, when, in terms of homogeneity, the implementing decree specifies an incorporation of 2 kg/t, or even 0.05 kg/t.
  - Risk accidental carry-over from another line or from residues of the drug substance if used by the plant on a regular basis.

3. External tracers

3.1. Pros

- There are no grounds for suggesting that an internal tracer is more representative of other mix constituents than an external tracer.
- Concerning industrial process performance assessments that use powders to assess homogeneity or carry-over, it is the physical behaviours that are assessed in relation to chemical analyses of the samples. The characteristics of analysis performance thus play a key role in tracer selection. External tracers can therefore be selected based on these criteria in order to obtain a reliable measurement of the mechanisms involved.
- The low analysis cost allows industrials to perform more frequent studies on process validation and factors. This process makes for safer production processes.
- A product's physical behaviour may be comparable to that of another product that differs in terms of its physical properties, if the external factors (matrix, industrial practices, etc.) have a greater impact on the assessed behaviours.
- It may be selected based on the number of particles, a key criterion in homogenisation assessments.
- With certain external tracers, the size of the processed sample can be adapted to fit the animal's feed intake that, according to current thinking, should be the feedstuff fraction for which homogeneity has to be guaranteed.
- This type of tracer does not require any in-plant recycling.
- The tested feedstuff may be the one that is most representative of plant production.
- It is easy to find four successive batches of the same size for use in carry-over tests. Incorporating the tracer into the first two batches and their presence, due to carry-over, in the following two batches, has no effect on industrial practices.

3.2. Cons

- At present, there are few external tracers available (microtracer, methyl violet, cobalt). Most of the findings at Tecaliman concern the RF-blue lake microtracer, which does not give an overall view of the issue.
- With the possible exception of cobalt, used in the Netherlands, there is no external tracer that currently guarantees reliable results following pelleting (microtracer, methyl violet). Tecaliman is actively researching the use of microtracer in this case.
- The use of cobalt as a tracer (Netherlands) means that tracer batches have to be recycled due to the "toxicity" of the concentrations involved.
- The use of a microtracer requires the absence or removal of all magnetised elements on the tested circuit, despite there being no actual evidence of their effect.
- Care has to be taken to avoid disturbing external tracers by the incorporation of liquids that would either limit their pre-analysis extraction potential, or cause them to migrate into the liquid phase, meaning that the tracers would no longer be representative of powder behaviour.

4. Conclusion

The general question that arises is primarily based on the fixed test objective. If, in accordance with Directive 95/69, the objective is to test the performance of a given installation, external tracers may be used and, in many cases, may even improve feed safety. Therefore, for over twenty years now, based on the criteria for detection thresholds, analytical precision, absence in raw materials and consistency of the physical properties of the baseline product, Germany has chosen to use an external tracer: methyl violet.

If the aim is to test the dispersion of a given product, then this product should be used, but the test conclusions may not be generalised to include mixer performance, as poor dispersion could be due to the product itself.

Lastly, other external tracers may be chosen as a baseline product in the future, as Tecaliman is currently participating in a European project on this topic.

5. Bibliographic references

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